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REACTION OF GALL BLADDER TO STIMULATION OF GASTRO-INTESTINAL TRACT

I. Response to Substances Injected into the Duodenum

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The two articles herewith presented are a natural continuation of a former study dealing with an "analysis of the reaction of the human gall bladder to food" (1) and represent an attempt to secure more specific data regarding the mechanism by which the musculature of the gall bladder is activated. The first section of the work deals primarily with the response of the human gall bladder to substances other than food,—i.e., to reagents applied directly to the duodenal mucosa through a Rehfuss tube. The second section deals with the response of the gall bladder in experimental animals to faradic stimulation of the splanchnic musculature, and affords conclusive proof that the gall bladder is under control of reflexes originating in the gastro-intestinal tract.[1]

The methods employed in recording the reactions of the human gall bladder were similar to those used in previous studies. X-rays of gall bladders that had been visualized by oral administration of tetraiodophenolphthalein were taken at short intervals before and after a given substance was introduced into the duodenum. Then the changing volumes were computed and plotted on coördinate paper against the time intervals. (For details of this method, see appendix to article previously cited (1).) The subjects used were medical students, from many of whom two series of x-rays were obtained at intervals of a few months, thus providing additional checks as well as opportunities for comparing the effect of different reagents on the same individual. During the course of each experiment the contents of the duodenum were continuously siphoned and frequently titrated, so that the charts give a simultaneous record of quantitative

[1] For preliminary abstracts see Proc. Soc. Exp. Biol. and Med., xxv, 840; xxvi, 466.

changes in the volume of the gall bladder and of the changing acidity and alkalinity of the duodenum. The latter is especially important since Ivy's cross-circulation experiments (2) would lead one to expect that sudden spurts of gastric juice into the duodenum might induce emptying of the gall bladder. But, as will be noted by detailed inspection of each chart, it is only occasionally that alternating changes in pH of the duodenum seem to influence the gall bladder. Perhaps this is due to the small quantity of fluid involved in these changes, or to the brief time that elapses before acid contents of the duodenum are neutralized (cf. p. 290).

STANDARD OF MEASUREMENT. At the conclusion of each experiment, the student was given a meal of egg-yolk to test the motility of the gall

TABLE 1

Reaction to one egg-yolk

CASE	DURATION	AMOUNT BILE DISCHARGED	PERCENTAGE	BLADDER BILE FIRST ASPIRATED
	minutes	*cc.*		*minutes*
W.F., fig. 3	26+	18	67	10
J.B.G., fig. 2	32+	10	74	9
M.K., (1) fig. 2	32+	23	77	14
E.M.L., (1) fig. 1	10+	6.5	87	7
E.M.L., (2) fig. 1	16	24	51	11
R.M., (1) fig. 2	16+	9	82	8
E.D.P., fig. 3	40+	15	65	15
M.M.S., fig. 3	30	26	91	12
H.A.S.*	22	19	73	—
A.D.W., (1) fig. 1	20	46	84	8
A.D.W., (2) fig. 1	38	59	84	13
T.J.W., fig. 3	32	15	65	21
Average	26+	22.5	75	11.6

* Figures 2 and 3 (Boyden and Saunders, 1928).

bladder and to afford a standard of comparison with other individuals. Egg-yolk was chosen because it has been established as the most effective means of emptying the biliary reservoir, and because more quantitative data with regard to its action on the gall bladder have been accumulated than is true of any other food or reagent (1). The amount of fluid injected into the duodenum through the Rehfuss tube, per experiment, was approximately 30 cc. This standard volume was adopted in order to secure uniformity of mechanical conditions and because it was large enough to be effective without unduly distending the bowel. In the case of egg-yolk, the 30 cc. injected consisted of one yolk well mixed with water. As pointed out in a previous article, this amount of egg-yolk induces but one phase of contraction in the gall bladder, yet this phase is quantitatively as great

if not greater than the first phase induced by five yolks taken by mouth,—the difference being that in the latter case successive spurts of yolk from the stomach induce successive phases of contraction which finally result in complete evacuation of the gall bladder.

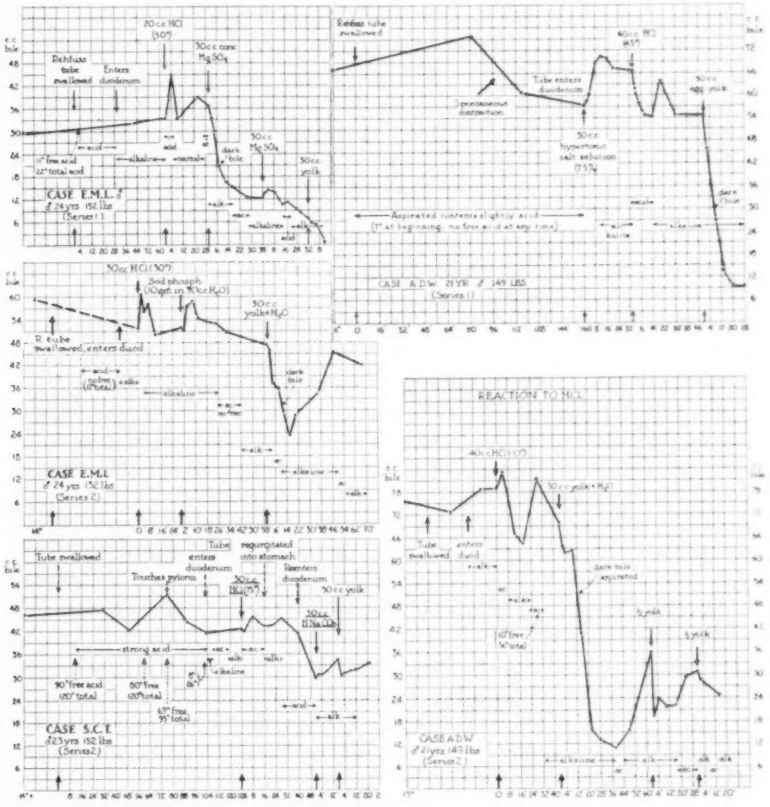

Fig. 1. Graphs recording change in volume of human gall bladder after injections of HCl and other reagents into the duodenum. Abscissas represent time in minutes after injection; ordinates, cubic centimeters of bile in fundus and body of gall bladder. Notations under the curve give changing acidity and alkalinity of duodenum as determined by testing aspirated contents with litmus paper, or by titrations. Series 1 and 2 separated by interval of two months or more.

Table 1 contains a list of twelve out of fourteen cases in which 30 cc. of egg yolk were injected into the duodenum. (Cases *C.S.S.*, fig. 4; and *S.C.T.*, fig. 1, are omitted as being obviously atypical.) This table shows

that one yolk thus administered causes a phase of contraction of the gall bladder which averages 26 minutes in duration and expels three-quarters of the contents of the gall bladder. Apparently it takes from seven to fifteen minutes for the head of the column of bladder bile to reach the duodenum after egg yolk is placed in the duodenum.

MECHANICAL STIMULATION. In a previous article (3) it was shown that sudden inflation of the human duodenum with air caused an initial discharge of approximately 4 cc. of bladder bile and, in one patient, the opposite effect—inhibition and filling of the gall bladder. These reactions to pressure changes in the duodenum were considered strong experimental evidence of the existence of reflex arcs extending from the duodenum to the gall bladder. In view of these experiments we were interested, therefore, in seeing whether the mechanical irritation of the Rehfuss tube in either stomach or duodenum would induce any changes in the volume of the gall bladder. As the length of time that the tube remained in the stomach prevented the taking of very many x-rays, there are no detailed data showing the initial reaction to the presence of the tube, but in three cases marked spontaneous emptying of the gall bladder (16 to 18 cc. of bile) occurred during the time the tube was in the stomach (cases *A.D.W.* (1), fig. 1; *S.C.T.*, fig. 1; and *T.J.W.*, fig. 3. In a fourth case there was filling to the extent of 18 cc. *J.B.G.*, fig. 2; and in a fifth, no change at all (*M.K.* (2), fig. 2). Two possible interpretations present themselves. In view of the experiments dealing with faradic stimulation of the stomach (part II, p. 302) we are inclined to postulate that these spontaneous contractions of the human gall bladder are due to rhythmic hunger contractions of the stomach. A second possibility that presents itself is that such spontaneous emptying of the gall bladder is due to spurts of chyme passing into the duodenum as a result of the irritating effect of the tube on the gastric mucosa. One objection to this is that the changes in volume are rather large for this reagent, as indicated below. Another objection is that the gall bladder may show marked contraction as the Rehfuss tube passes into the duodenum, even though the aspirated contents are alkaline (*J.B.G.*, fig. 2).

HYDROCHLORIC ACID. In testing the effect of hydrochloric acid we have confined the dosage injected into the duodenum to strengths usually occurring in the normal stomach, i.e., to amounts not exceeding 50 or 75 degrees.[2] The results of eight trials are shown in table 2. In four cases there was immediate contraction and diminution of the gall bladder volume followed by filling, the apparent decrease in volume ranging from 3 to 32 per cent. But in no case was any bladder bile aspirated from the duodenum following injection of hydrochloric acid. This indicates that we

[2] One degree represents the number of cubic centimeters of N/10 NaOH needed to neutralize 100 cc. of gastric contents.

are dealing with discharges of bladder bile which are so small in volume as to merely fill the bile passages. This seems the more probable when we consider the shortness of the period of contraction induced by injections of hydrochloric acid, viz., 8 to 14 minutes. For this is within the range of time that it takes the head of the column of bladder bile to reach the duodenum after ingestion of egg-yolk. So that it would appear that the ineffectiveness of HCl is due to the fact that it cannot sustain contraction of the gall bladder long enough to force bladder bile into the intestine. Evidence for this is twofold: 1st, that acid introduced into the duodenum by Rehfuss tube is neutralized in from 4 to 16 minutes (in the seven cases in table 2, the average was 9 minutes); and 2nd, that natural spurts of

TABLE 2

Hydrochloric acid

CASE	AMOUNT	STRENGTH	TIME FOR NEUTRALIZATION	REACTION OF G. BL.	DURATION	VOLUME CHANGE	PER CENT CHANGE
	cc.	*degrees*	*minutes*		*minutes*	*cc.*	
B.B., fig. 6	40	40	5	Contraction	8	2.5	9
J.B.G., fig. 2	40	40	8	Contraction	8	9	22
A.D.W. (1), fig. 1	40	43	14	Contraction	14	12	18
A.D.W. (2), fig. 1	40	10	8	Contraction	14	19	23
Average					11	10.6	18
S.C.T., fig. 1	30	75	16	Filling	8	4	9.5
E.M.L. (2), fig. 1	30	30	4	Filling	2	9	17
E.M.L. (1)	20	50	6	Filling	4	11	3
H.A.S.*	30	25	—	Filling	22	20	32
Average			9		9	11	15.4

* Figure 2, Boyden and Saunders (1928).

acid chyme from the stomach, although tolerated longer, are neutralized in approximately 13 minutes (average of 33 instances in which acid was aspirated from the duodenum, figs. 1 to 6). This brief reaction of the gall bladder to hydrochloric acid is in marked contrast to its response to food and to certain hydragogue cathartics.

SALINE CATHARTICS. From the standpoint of their effect on the gall bladder the cathartic salts may be divided into three groups: 1, those that induce marked emptying of the gall bladder; 2, those that are erratic but at best produce only moderate emptying; and 3, those that regularly cause an initial relaxation or inhibition of the gall bladder instead of emptying.

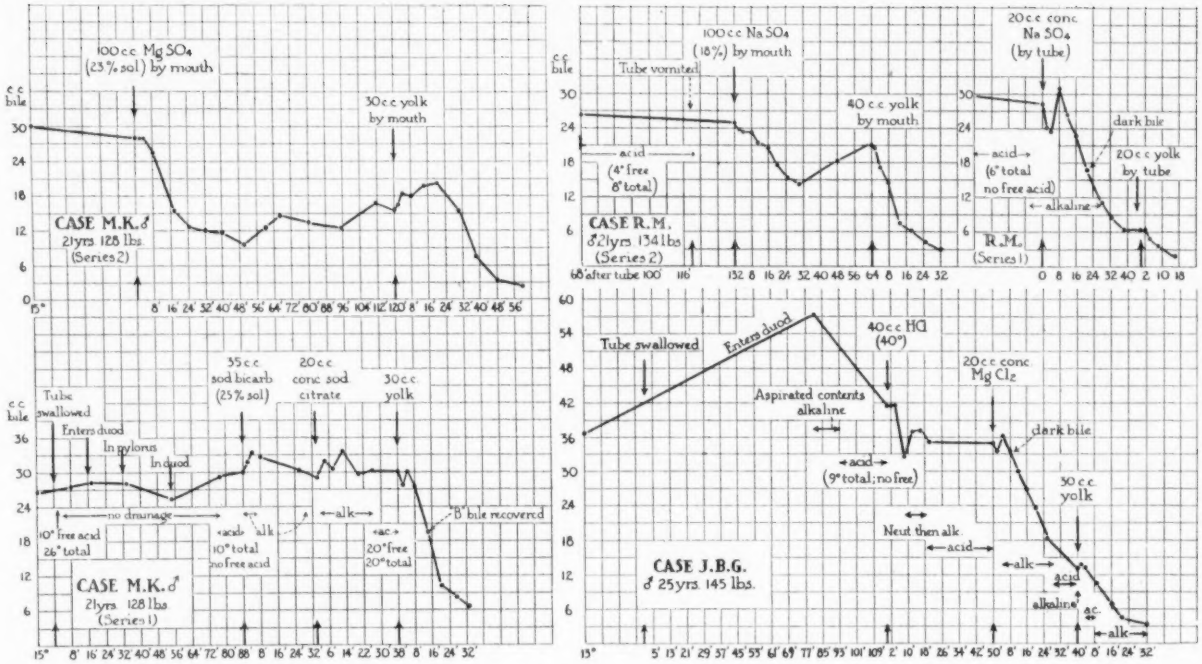

Fig. 2. Graphs recording change in volume of human gall bladder after injecting hydragogue cathartics and other reagents into the duodenum. In two cases, *M.K.* (series 2) and *R.M.* (series 2), the salts were taken by mouth.

TABLE 3

REAGENT	NAME	AMOUNT	STRENGTH	ACTION	DURATION	VOLUME CHANGE	PER CENT CHANGE
			A. Effective cathartics				
		cc.					
$MgSO_4$ (epsom salts)	B.B., fig. 6	40	Saturated solution	Contraction	40	10	31
	D.S.*	30	Saturated solution	Contraction	16	19	39
	M.K. (2), fig. 2	100	23 per cent solution by mouth	Contraction	50	18	64
	E.M.L. (1), fig. 1	30	Saturated solution	Contraction	38	25	67
	H.A.S. (1)*	30	Saturated solution	Contraction	32	41.5	66
$MgCl_2$	J.B.G., fig. 2	20	Saturated solution	Contraction	40	23	64
Na_2SO_4 (Glauber's salts)	R.M. (1), fig. 2	20	Saturated solution	Contraction	40	24	77
	R.M. (2), fig. 2	100	18 per cent solution by mouth	Contraction	30	11	44
			B. The tartrates				
Rochelle salts (K and Na tartr.)	H.F.O., fig. 3	12 grams in 100 cc. H_2O by mouth		Contraction	40	19	33
	E.D.P., fig. 3	10 grams in 30 cc.		Contraction	26	9	38
Sod. tart.	W.F., fig. 3	8 grams in 30 cc.		Contraction	20	12	36
Rochelle salts	M.M.S., fig. 3	10 grams in 30 cc.		Filling	16	13.4	50
Sod. tart.	T.J.W., fig. 3	10 grams in 30 cc.		Negative	—	—	—
			C. Other sodium salts				
Sod. chloride	A.D.W. (1), fig. 1	30 cc. 7.5 per cent solution		Filling	12	13	23
Sod. bicarbonate	M.K. (1), fig. 2	35 cc. 25 per cent solution		Filling	4	35	12
	S.C.T., fig. 1	30 cc. 25 per cent solution		Filling	8	4	9.5
Sod. phosphate	E.M.L. (2), fig. 1	10 grams in 30 cc. H_2O		Filling	6	7.6	14
Sod. citrate	M.K. (1), fig. 2	20 cc. saturated solution		Filling	12	5	17

* Figure 2, Boyden and Saunders.

First group ($MgSO_4$, $MgCl_2$, and Na_2SO_4). A study of table 3-A shows that in five out of eight cases the gall bladder emptied approximately two-thirds of its contents within the first forty minutes after the salts were injected into the duodenum. In two of the cases (*M.K.* (2), and *R.M.* (2), fig. 2) the salt was administered orally, the data suggesting that it is not quite so effective when given by mouth (cf. cases *R.M.* 1 and 2). Finally, dark bile was aspirated in the four cases in which a reduction of over 60 per cent was noted in the volume of the gall bladder. In two cases the head of the column of bladder bile reached the duodenum in eight minutes (*dark bile*, *E.M.L.*, fig. 1; *J.B.G.*, fig. 2). These observations make it quite clear that these three cathartic salts induce an emptying of the gall bladder that is equivalent in every respect to the first phase of contraction induced by a meal of egg-yolk.

Second group (Sodium and potassium tartrate). Three out of the five cases in this group (table 3-B) showed a moderate reaction to the salt, not more than one-third of the contents of the gall bladder being emptied in response to its presence in the duodenum. Apparently oral administration of it was as effective as by duodenal tube. Not only were the volume changes and the duration of its effect less than that of the first group but the aspirated bile was noticeably lighter in color. Perhaps the lesser effectiveness of the tartrates was due to the fact that on account of their greater toxicity they had to be administered in weaker solution than group I.

Third group (Other sodium salts: chlorides, bicarbonates, phosphates, and citrates). The effect of these salts in all five cases was to induce an initial though temporary inhibition or relaxation of the gall bladder (table 3-C).

Discussion. One of the oldest methods of treating cholelithiasis is the so-called Karlsbad treatment which, it is said, depends for its value upon two things: The scientific dieting of the patient, and the occurrence of cathartic salts in the Karlsbad Sprüdelwasser. According to Hemmeter (4), this treatment has been carried on at Karlsbad since the year 1347. He himself, having there observed the effect of $MgSO_4$ and other salts on the stools, was able in 1895 to provoke a flow of bile by injecting Karlsbad water into the duodenum (incidentally, this is said to have been the first recorded use of a duodenal tube in patients). Since 1919 the efficacy of $MgSO_4$ in evacuating the gall bladder has been extensively advocated by Lyon, but as vigorously denied by others. The introduction of the Graham method, however, has made it possible for the first time to measure the reduction in size of the gall bladder after $MgSO_4$ (3), and to demonstrate quantitatively that injection of any of three cathartic salts in group I produces nearly as great an initial emptying of the human gall bladder as that caused by a meal of egg-yolk.

The difficult problem at present is to explain the mechanism by which salts in the duodenum can activate the musculature of the gall bladder. (Obviously they also open the intestinal orifice of the bile duct or the bile could not escape.) Two sorts of explanations have been advanced. One of these would attribute the evacuation of the gall bladder to the increased peristalsis of the intestine following mechanical distention provoked by

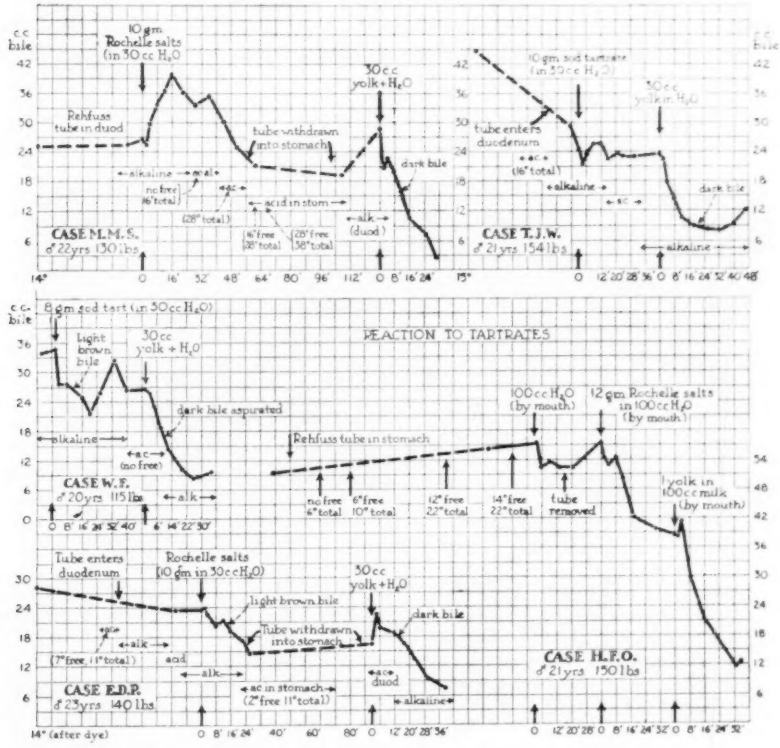

Fig. 3. Graphs recording change in volume of human gall bladder after duodenal administration of sodium and potassium tartrates (Rochelle salt), and other reagents.

the osmotic action of the unabsorbed salt, the latter causing an inflow of tissue fluids which continues until the contents of the intestine are isotonic with the blood. Although the earlier theory that this accelerated peristalsis would "milk" the bile into the intestine is no longer tenable it is still possible, in view of the evidence presented in the second section of this paper, that peristalsis may induce reflex contraction of the gall bladder.

But on this basis hypertonic solutions of $NaCl_2$ should also empty the gall bladder, since they distend the bowel and are even said to evoke a "peristaltic rush" when placed in the duodenum (5). Yet just the opposite happens (*A.D.W.* (1), fig. 1).[3] Also from the standpoint of distention of the bowel, other hydragogue salts should be as effective as those in group I. In the hope of solving this problem we turned to animals for more extensive experimentation but found to our surprise that when $MgSO_4$ was injected into the duodenum of cats it had no effect on the gall bladder. Similar negative findings in dogs have been reported by Gantt and Volborth (7).

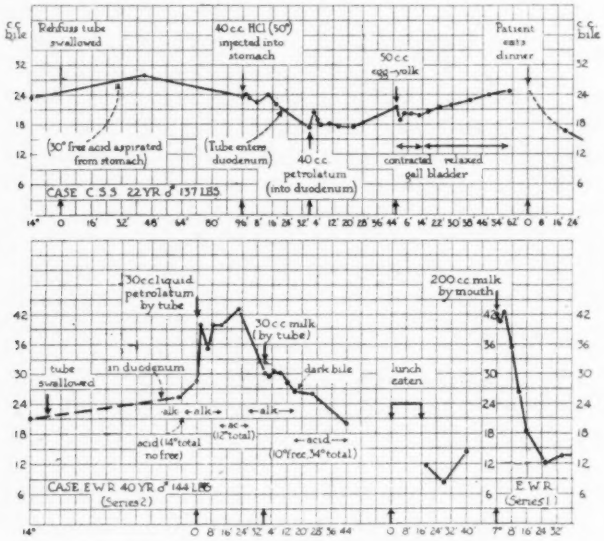

Fig. 4. Graphs recording change in volume of human gall bladder after duodenal injection of liquid petrolatum and its depressant effect upon the action of foods subsequently administered.

As a consequence one must await more specific information regarding the relative effect of different salts on peristalsis of the human intestine, before disposing of this theory.

A second explanation would attribute evacuation of the gall bladder to

[3] In this connection Cole's observations (6) are of special interest. He found that saline irrigation of the stomach raised the pressure in the common duct 10 to 30 mm., and that concentrated sodium bicarbonate caused a spasm of the papilla. As the latter substance causes relaxation of the gall bladder (*M.K.* (1), fig. 2; *S.C.T.*, fig. 1), a reciprocal action of gall bladder and sphincter appears to have been brought about by the same reagent.

reflexes arising from direct stimulation of nerve endings in the intestine, thereby implying the direct action of ions in these salts. But when we try to select the effective ions by elimination, an impasse is soon reached unless it be assumed that the action of an effective ion is sometimes masked by its co-ions, or that several ions are equally active. Thus Na_2SO_4, for instance, is effective, but that is not true of any other sodium salt. Consequently one assumes that it must be the sulphate ion in Na_2SO_4 that is active. When we examine the chlorides, it appears that $MgCl_2$ evacuates the gall bladder but that NaCl has the opposite effect so that in this reagent it must be the magnesium ion that is effective. This process of elimination leaves us with both the magnesium and the sulphate ions. Yet both of these are said to be the least absorbable, hence their combined

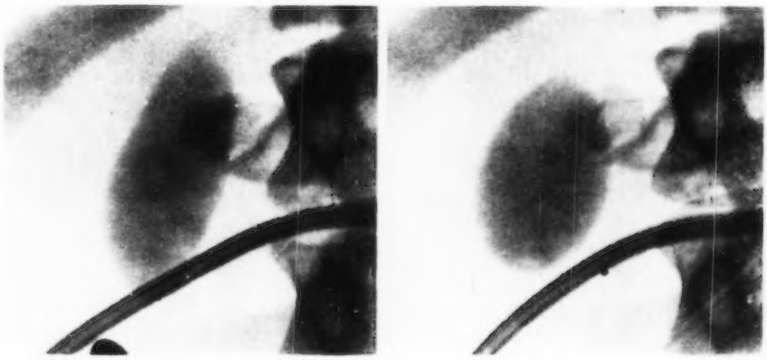

Fig. 5. Cholecystograms from case *C.S.S.*, figure 4, showing condition of gall bladder at 8 and 23 minutes, respectively, after egg-yolk. In the first picture, it is in strong tonus but cannot expel its contents (cf. fig. 4); in the second picture, it is relaxed.

value as an osmotic cathartic (Sollmann). Furthermore, when absorbed, magnesium is said to depress all nervous and muscular functions even in very dilute amounts. For the present, therefore, no satisfactory solution of this problem seems to be available. Yet $MgSO_4$ induces the most powerful evacuation of the gall bladder exerted by any inorganic reagent.

In concluding these observations on $MgSO_4$, a word should be said about the duration of its action. Empirically, we know that its effect on the gall bladder is completed within forty minutes. This is reasonable in view of the dilution and chemical changes which it undergoes, and the speed with which it travels through the intestine,—appearing in the stools in three hours (Ury, in Sollmann). It is obvious, therefore, that x-rays taken three or four hours after administration of $MgSO_4$—as in Lyon's recent article (8)—have no significance but rather, indeed, tend to discredit its efficacy.

PETROLATUM. In addition to the hydragogues listed above, it was planned to test the action of several other types of cathartics. Among these was liquid petrolatum. In the two cases in which this was tried, its immediate action on the gall bladder was inhibitory (fig. 4). The most interesting feature was the effect it seemed to have on subsequent injections of food. Thus, in case *E.W.R.*, figure 4, it slowed down the normal action of milk in evacuating the gall bladder. The same was true with egg-yolk (case *C.S.S.*, fig. 4). But after the duodenum had been scoured

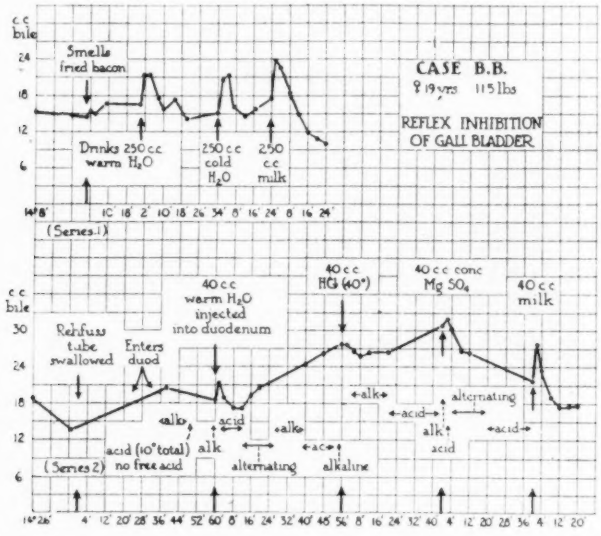

Fig. 6. Graphs comparing change in volume of human gall bladder after ingestion of fluids by mouth with change after injection of fluids into the duodenum. In both series, the gall bladder of this individual responded to the presence of water or milk in the stomach or duodenum by an initial relaxation. The two series were taken several months apart.

by a meal of solid food, evacuation of the gall bladder proceeded normally. A possible clue to this situation is afforded by x-rays of case *C.S.S.* which show that after egg-yolk the gall bladder was in strong tonus (fig. 5) but that it was unable to expel its contents, since its volume remained constant. This recalls the similar behavior of an hour-glass gall bladder presented in a previous publication (1), and suggests that petrolatum acts locally in closing the sphincter of Oddi but does not prevent contraction of the gall bladder in response to the presence of food in the duodenum.

REFLEX INHIBITION OF THE HUMAN GALL BLADDER. In presenting the

last of these cases, attention is called to a pair of experiments which, as in the case of the distention of the duodenum with air, suggests the existence of inhibitory reflexes originating in the duodenum (fig. 6). In the first series the young woman was given three successive glasses of water or milk to drink, and in each case sudden relaxation of the gall bladder ensued. (For x-rays of this case see fig. 2, (9).)

In the second series, smaller amounts of water and milk were given by duodenal tube, and evoked an equally sudden relaxation of the gall bladder, indicating that if the first effect was a reaction of the stomach, the second, at least, was due to stimulation of the duodenum. But in either series the suddenness of the reaction and its transitory character made it seem probable that we were dealing with inhibitory reflexes arising from the splanchnic area. It was reflection upon this feature of these experiments that led us to undertake direct faradic stimulation of the gastro-intestinal tract, as outlined in the following section of this paper.

SUMMARY

1. One egg-yolk injected into the duodenum induces a single phase of contraction of the gall bladder and evacuates three-quarters of its contents. The head of the column of bladder bile may be aspirated from the duodenum seven to fifteen minutes after the injection of yolk.

2. Injections of strong solutions of $MgSO_4$, $MgCl_2$, or Na_2SO_4 evacuate two-thirds of the contents of the gall bladder, the column of bile reaching the duodenum as quickly as after egg-yolk. When given by mouth these salts are nearly, if not as, effective as when given by Rehfuss tube.

3. Rochelle salt, or one of its constituents (sodium tartrate) never empties more than a third of the contents of the gall bladder and is not consistent in its action. The aspirated bile is a lighter brown than the usual "B" bile.

4. Other sodium salts, such as the common chloride, bicarbonate, phosphate and citrate, induce temporary relaxation and filling of the gall bladder instead of emptying.

5. Injection of hydrochloric acid, in strengths found in the normal stomach, induces moderate changes in volume of the gall bladder (less than that caused by the tartrates); and may be in either direction. But its action is transitory and when positive never results in the aspiration of recognizable quantities of bladder bile. Alternating changes in the pH of the duodenal contents seem to have no appreciable effect on the tone of the gall bladder.

6. Injection of liquid petrolatum causes initial inhibition of the gall bladder and retards its response to food subsequently injected into the duodenum. The latter effect is thought to be due to the local action of petrolatum in closing the sphincter.

7. The simultaneous contraction of the gall bladder and opening of the sphincter after egg-yolk or $MgSO_4$, together with the synchronous relaxation of the gall bladder and closing of the sphincter (Cole) after sodium bicarbonate, implies the existence of a reciprocal mechanism which is set in motion by a single reagent.

8. Sudden contraction of the gall bladder following distention of the duodenum with air, or sudden relaxation (in certain individuals) accompanying the injection of fluids, suggests that the human gall bladder is subject to reflexes arising in the gastro-duodenal tract,—a theory which is confirmed by experiments on animals, as recorded in the companion article to this paper.

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REACTION OF GALL BLADDER TO STIMULATION OF GASTRO-INTESTINAL TRACT

II. Response to Faradic Excitation of Stomach, Small Intestine and Cecum

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As a result of the evidence presented in section I of this article, which strongly suggests that the human gall bladder is under the control of the nervous system and that the same stimulus that opens or closes the end of the common bile duct also causes contraction or relaxation of the gall bladder, we undertook to devise a method by which the ampulla of Vater could be stimulated directly without employing any form of anesthesia.

For this purpose the experiment illustrated in figure 1 was devised. The cats were laparatomized in the morning. During the operation two small rubber tubes were inserted into the common bile duct. One of these, the excurrent tube (see arrows), was designed merely to drain the bile from the hepatic ducts to the exterior. The other, the incurrent tube, contained two enamelled copper wires leading from an induction coil to the cannula in the ampulla of Vater. The wires were completely insulated by enamel except at the tip ends which projected into the ampulla. Before closing the body cavity permanently the gall bladder was filled with iodized oil (according to the method of Whitaker) and the current was tested in the anesthetized animal. It was observed that even a weak stimulus (half a milliampere) induced a local ring contraction of the duodenum at the level of the ampulla. The evening of the same day, when the cat was in good condition, purring and moving about, it was placed on the x-ray table and connected with the induction coil and batteries. In addition to testing the current at the time of operation, its effectiveness during the experiment was recorded in two ways, by an ammeter and by the reaction of the cat,—the current temporarily disturbing the animal so that it cried as if troubled with a colic-like pain.

Faradic stimulation of ampulla of Vater. Plate 1 consists of a series of x-rays showing the inhibitory effect on the gall bladder of stimulating the choledochal segment of the duodenum with an induction current. (For orientation, compare with sketch in fig. 1.) The first picture at the

top of the plate shows the gall bladder contracting spontaneously eight hours after the operation. Application of a weak current ($\frac{1}{2}$ milliampere) causes relaxation of the gall bladder ($1\frac{1}{4}'$), as indicated by its greater width and the slumping of the column of iodized oil. The same inhibition may be induced when the gall bladder has regained its tonus and is emptying following ingestion of egg yolk (*15' pc.* and 2' and 3' after current). The last picture (*38' pc.*) shows the gall bladder again emptying through the drainage tube, under the influence of yolk.

Since the testing of the current at the time of operation revealed the fact that even the weakest current caused deep contraction of that segment of the gut that lies at the level of the ampulla, and that it was not possible

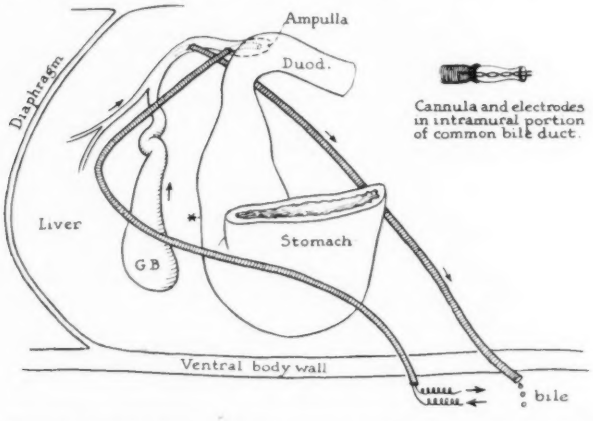

Fig. 1. Sketch showing method of intubation used in first experiment; and organs of cat in situ, as viewed from the left side. *G.B.*, gall bladder; *stomach*, inferior cardiac portion, with upper part removed to expose pars pylorica and duodenum; *asterisk*, position of electrodes in second experiment (on greater curvature of pars pylorica). *Cf.* with position of terminals in plates 1 to 4.

by this method to stimulate the mucosa without causing contraction of the intestine, the experiment was simplified by discarding the double intubation of the common duct and merely sewing the rubber tube containing the enamelled wires to the peritoneal surface of the gut tract. This was done in such a way that the exposed terminals were in contact with the peritoneum but were completely insulated from adjacent organs. The first segment to be studied in this way was the pyloric part of the stomach (*asterisk*, fig. 1).

FARADIC STIMULATION OF STOMACH. *Spontaneous rhythm of gall bladder.* When previously working with cats in which the gall bladder had been filled with iodized oil, we had occasionally noted spontaneous changes in

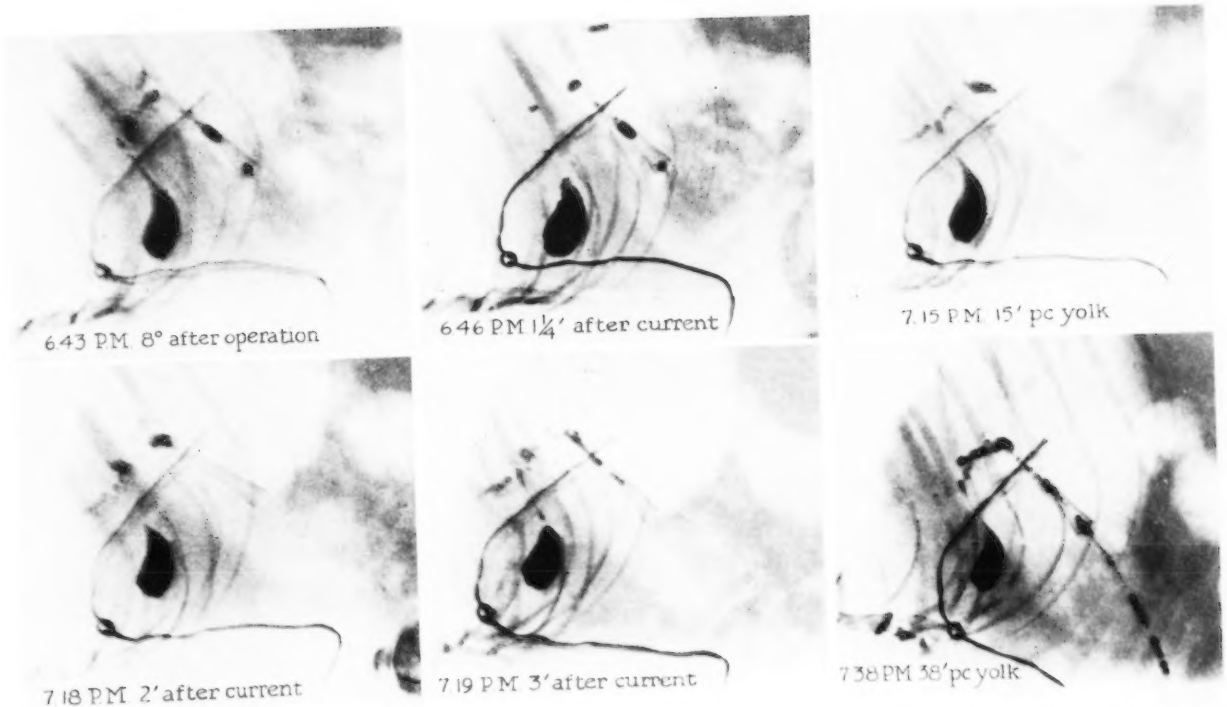

Plate 1. Reaction of contracting gall bladder to faradic stimulation of intraduodenal portion of common bile duct (for orientation, cf. fig. 1).

the tonus of the gall bladder during fasting. Since these occurred while the cat was resting quietly on the x-ray table, they could not be attributed to mechanical factors. But it was not until the present investigations were begun that the significance of these changes became clear. In order to differentiate between such spontaneous changes in shape and the response of the gall bladder to faradic stimulation of the stomach, the cat shown in plate 2 was photographed every half-minute for a considerable period; with the result that a natural contraction rhythm was revealed at intervals of one to three minutes (beginning at 7:05 p.m., 7:06½, 7:08½,

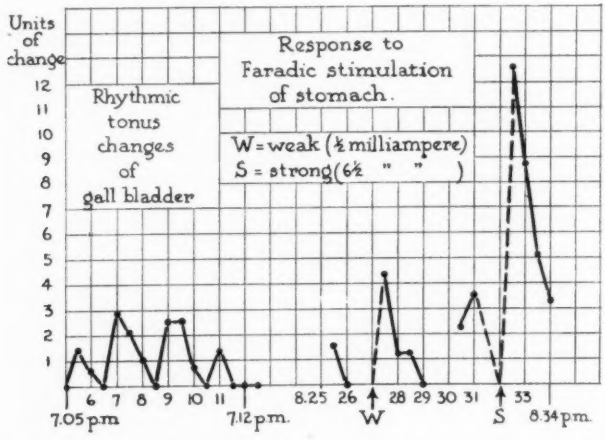

Fig. 2. Graphs showing relative amounts of oil forced into the upper lobe of the gall bladder in plates 2 and 3: 1, effect of rhythmic hunger contractions of stomach; 2, effect of weak faradic stimulation of pyloric stomach (½ milliampere); and 3, effect of summation of stimuli, the weak current (*W*) being followed by a strong one (*S*), 6½ milliamperes. The latter is greater than that caused merely by a strong current (cf. rows 1 and 3, plate 3). The volumes were computed by methods described for human gall bladder (Boyden, 1928; see part I of this article).

and 7:10½; and at certain subsequent periods not included in plate 2). Curiously enough the stomach of this cat was visualized by its natural content of air, so that when the films were developed it was seen that the tonus changes in the gall bladder paralleled a series of peristaltic waves that were passing over the cardiac end of the stomach. A third favorable condition was the fortuitous shape of the gall bladder—the body being separated into two lobes by an incisure—so that every time it contracted some of the iodized oil was forced beyond the incisure into the upper lobe (plate 2). By computing the changing volumes of oil in the upper lobe it was therefore possible to study the tonus changes quantitatively. These

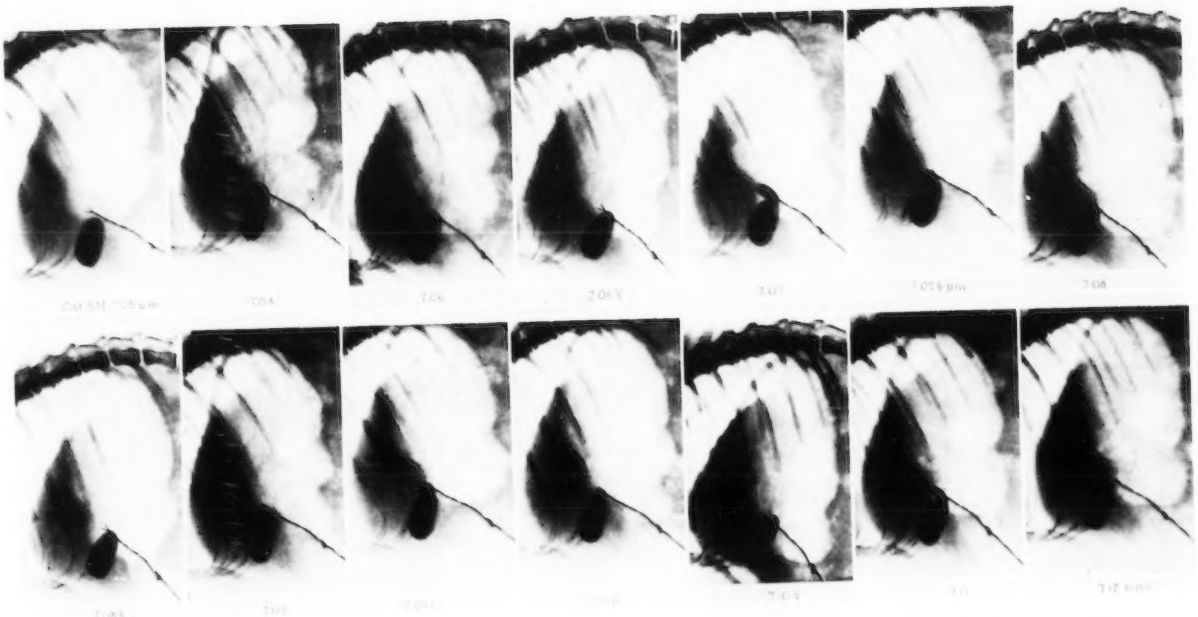

Plate 2. Synchronous contraction rhythms of stomach (white) and gall bladder (black) in fasting animal

results are shown graphically in figure 2. Subsequently, synchronous contractions of the gall bladder and stomach were noted in other cats.

Reflex contraction of gall bladder. Evidence that the spontaneous rhythm of the gall bladder just described was induced by peristalsis of the stomach, is indicated by the following experiment. When the pars pylorica of the stomach of the cat shown in plate 2 was stimulated with a strong induction current, the gall bladder immediately contracted, forcing the iodized oil further into the upper lobe than occurred under the influence of spontaneous contractions (first row, plate 3). Also when a weak current was applied to the stomach, the oil was again ejected but not so high as when the current was stronger (second row, plate 3; *W*, fig. 2). thereby revealing a quantitative relation between the strength of contraction of the stomach and that of the gall bladder. But when strong stimulation followed closely upon weak (third row, plate 3; *S*, fig. 2) there was a maximum response, the oil being shot high up into the cystic duct as if by contraction following summation of stimuli. Incidentally it may be noted that the greater the contraction, the longer is the time that is required for the gall bladder to regain its resting condition.

A second series, from another cat, is shown in plate 4. Here, even a weak current ($\frac{1}{2}$ milliampere) applied to the stomach ejected the iodized oil into the cystic duct, but the latent period was 30 seconds longer than when a stronger current was used (cf. rows 1 and 2). The pictures in this series were made from a cat with a double gall bladder and two cystic ducts, comparable to those described in a former publication (1). Only one of the gall bladders, however, had been filled with iodized oil, the cystic duct of the other having been tied off at the time of operation, leaving only a cul-de-sac on that side. But at first application of the tetanizing current, the gall bladder containing the iodized oil ejected its contents through its own cystic duct and backed up into the cul-de-sac representing the second cystic duct ($6.37\frac{1}{4}$, plate 4). There it remained during the rest of the experiment (see also plate 6). This indicates the strength of the contraction induced by reflexes from the stomach. In interpreting this series, it should be noted that the terminals are sewed to the greater curvature of the pars pylorica of the stomach which is on the right side, so that the resulting contraction of that part of the stomach is not visible. The pictures, however, show a bulging of the pars cardiaca immediately following stimulation, which, being on the side toward the observer, is visible. This bulging is evidently due to back pressure from the contracting pyloric end.

This experimental stimulation of the stomach seems to afford conclusive proof that vigorous contraction of the stomach induces reflex contraction of the gall bladder. In the case of the spontaneous rhythms shown in plate 2, the peristaltic waves of the stomach were not very deep and the changes in the tonus of the gall bladder were correspondingly moderate.

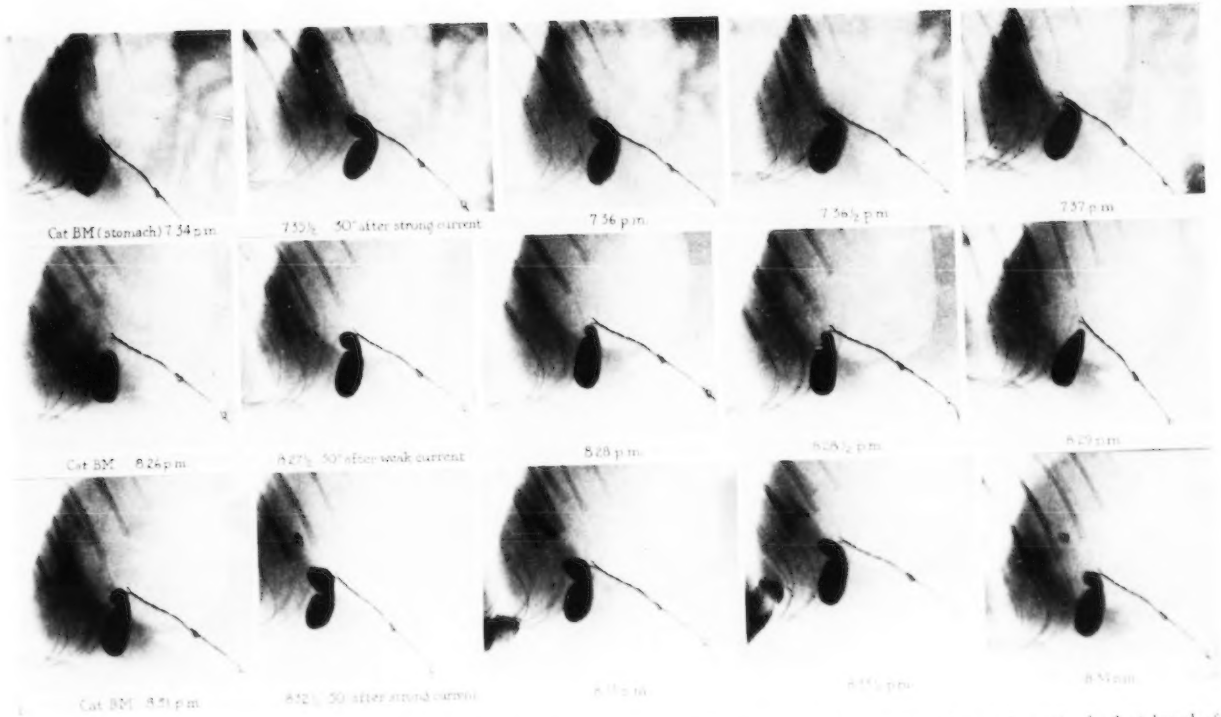

Plate 3. Reaction of relaxed gall bladder to faradic stimulation of pars pylorica of stomach (electrodes attached at level of asterisk, fig. 1).

But when strong currents were applied to the stomach, causing sudden and deep local contraction of the organ, then the iodized oil was ejected well into the cystic duct. When one considers than an induction shock lasting 10 to 20 seconds usually causes a temporary local contraction of the stomach it is not difficult to understand how a series of deep hunger waves occurring over a period of twenty minutes or half an hour, would empty the gall bladder of much of its contents. Such spontaneous contractions, during fasting, occur both in animals (plate 1) and in human beings (section I of this paper). In a human case already described, the patient reported intense, subjective hunger pangs at the time that the x-rays were recording pronounced emptying of the gall bladder (2). Previous to this Boldireff had shown that in dogs, periodic hunger contractions of the stomach are accompanied by spurts of bile from the common duct (3).

The time intervals of these contractions also make it evident that we are dealing with a rhythm superimposed upon the gall bladder by the stomach, and not with an intrinsic rhythm. For when a rabbit gall bladder is removed and placed in oxygenated Locke's solution, it manifests rhythmic contractions as rapid as 1 to 3 per minute (4). Other authors working with the gall bladder in situ have reported rates in dogs of from 2 to 5 per minute (5). These are not unlike the intrinsic rhythms of the small intestine (6), with a frequency of 6 to 11 or more per minute. Taylor and Wilson have shown that this intrinsic rhythm of the gall bladder is not synchronous with the intrinsic rhythm of the stomach, being sometimes greater and sometimes less (7). In marked contrast, therefore, are those much slower and more pronounced contractions of the gall bladder which are shown in plate 2 and figure 2 and which correspond in frequency to the hunger contractions of the stomach in man and animals as reported by various investigators.[1]

From a teleological point of view it seems rational that food taken into the stomach should start peristalsis of that organ, and that this in turn should induce contraction of the gall bladder, thereby releasing bile for the digestion of the food that is being ejected into the intestine by gastric peristalsis. Of special interest in this connection are the moving pictures recently demonstrated by G. M. Higgins of the Mayo Institute, showing simultaneous peristalsis of the gall bladder and stomach in fishes (9). While peristalsis of the gall bladder does not occur in mammals, we believe that this correlation we have established between the gall bladder and the stomach of the cat represents a significant persistence of this primitive vertebrate condition.

Mechanical tests. Since it might be said that the expulsion of bile from the gall bladder in the above experiments was merely due to some mechan-

[1] For latest data on hunger contractions of the human stomach, see Templeton and Johnson (8).

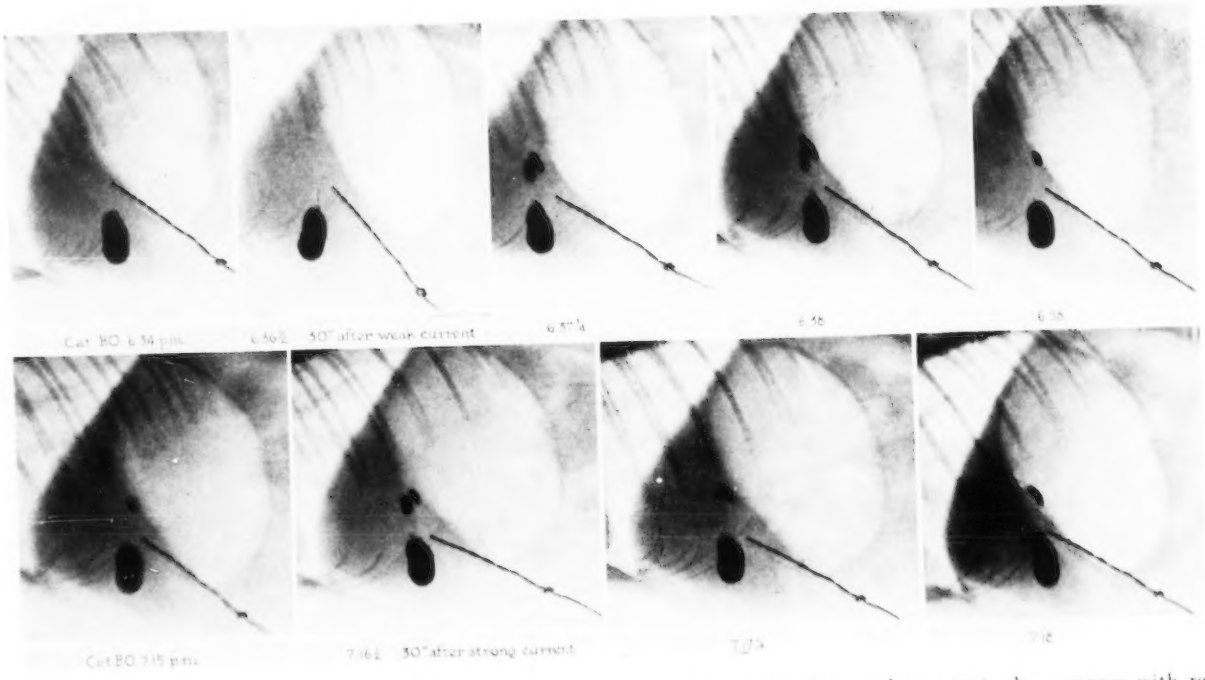

Plate 4. Reaction of double gall bladder to faradic stimulation of pyloric portion of stomach (see text, also compare with response of same gall bladder to current, after meal of egg-yolk, plate 6).

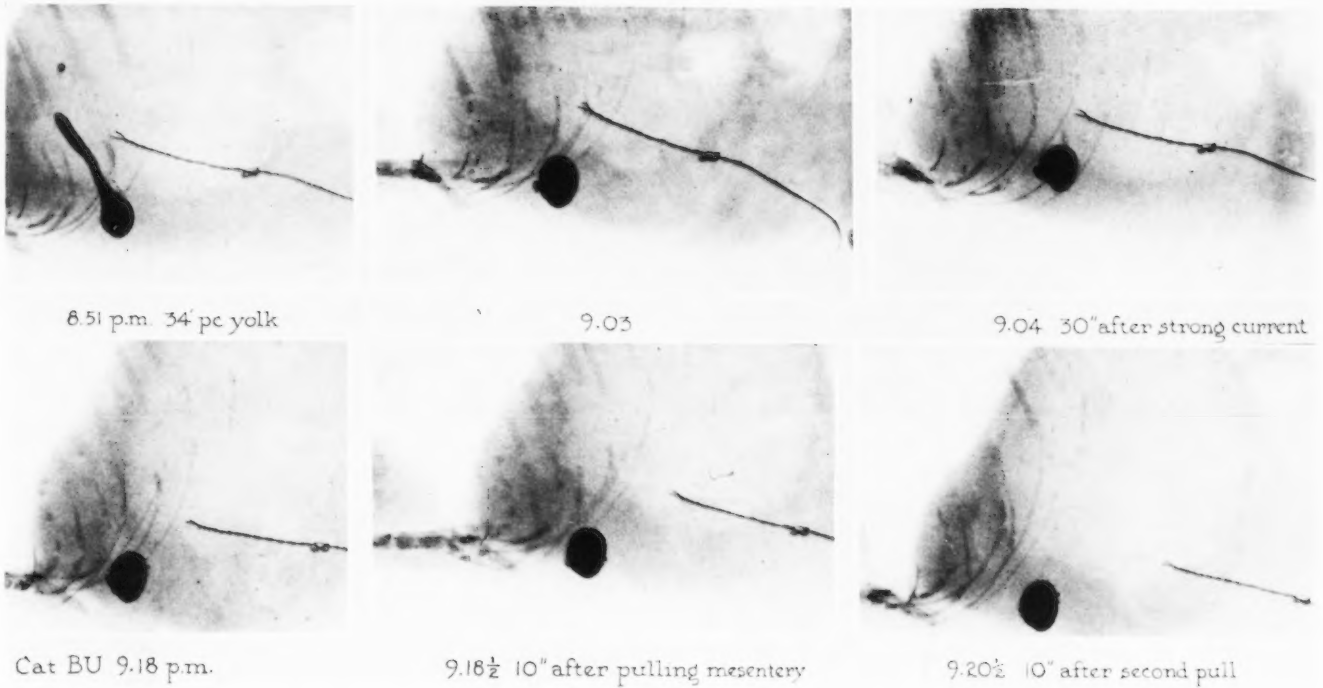

Plate 5. Test of mechanical factors. Electrodes sewed to pylorus; *8:51*, gall bladder momentarily under tonus after yolk; *9:04*, further relaxation after current; *9:18*, anesthetized animal; *9:18½*, *9:20½*, absence of response to mechanical pulling of pylorus (note changing position of electrodes).

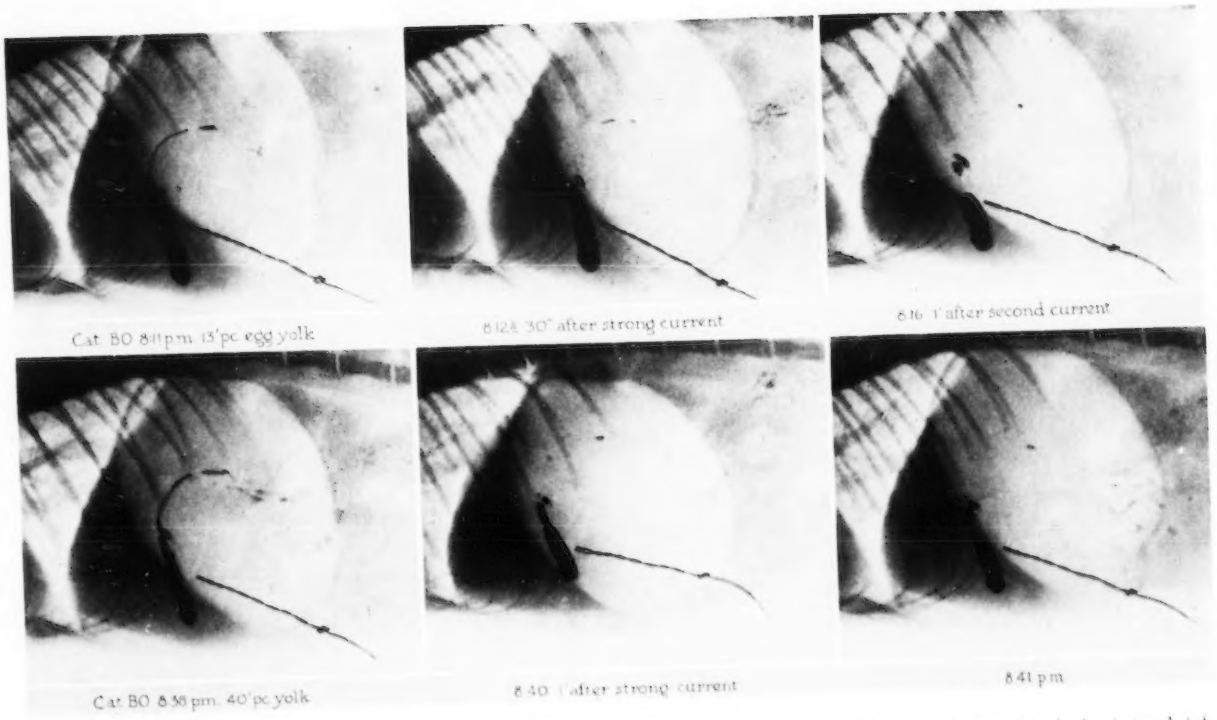

Plate 6. Reaction of gall bladder which is emptying under the influence of food, to faradic stimulation of pyloric stomach (cf. plate 4, from same cat).

ical effect, such as traction exerted by the contracting stomach on the mesentery leading to the liver and gall bladder, this possibility was tested in two cats by the following method. The rubber tube was sewed to the gut at the level of the pyloric sphincter and opposite the duodeno-hepatic ligament, so that if the portion of the tube outside the body were pulled, it would cause the most direct traction on the porta hepatis. At the time of the experiment numerous x-rays established the fact that there was a spontaneous rhythm of the gall bladder accompanying peristalsis of the stomach. Then the cat was fed egg-yolk. Apparently the irritation caused by sewing the tube to the pylorus caused a pyloro-spasm, for only once did any food get by the gate, judging from the solitary instance in which evacuation of the gall bladder was attempted (viz., *3½′ pc yolk*, plate 5). But this was sufficient to demonstrate that we were dealing with an active gall bladder. Thereupon the pylorus was stimulated with an induction current, which had the effect of relaxing the gall bladder still further (*30′* after current). Then the cat was placed under light ether anesthesia to abolish the reflexes to the gall bladder. After a preliminary picture (*9:18*), traction was exerted on the tube (compare position of terminals at *9:18* and *9:18½*). But the gall bladder failed to change its shape. A second pull (*9:20½*) produced a similar negative result, so that it may be concluded that mechanical shifting of adjacent organs is not a factor in the contraction of the gall bladder.

Reflex inhibition of gall bladder. Returning now to the experiments dealing with faradic stimulation of the stomach, it has been demonstrated (plates 3 and 4) that contraction of the stomach induces reflex contraction of the relaxed gall bladder. Realizing that the effect of a given stimulus might depend on the physiological state of the organ which is being tested, we next fed a series of animals, and then stimulated the stomach after the gall bladder had begun to empty. Application of a current to the pars pylorica of the stomach, under these circumstances, caused inhibition of the gall bladder (instead of contraction) and a dropping back of the column of iodized oil that had been supported by the gall bladder (plate 6). To appreciate the full significance of this, plate 6 should be compared with plate 4, both of which were taken from the same cat, during the same evening, with all factors the same except that in the second case the gall bladder was emptying under the stimulus of food. This reversal of effect recalls the observations made by Carlson and Litt, and others, on the reaction of the cardiac and pyloric sphincters to stimulation of the vagus nerve. When the sphincters are relaxed, excitation of the nerve causes contraction; but if they are already contracted, a comparable stimulus relaxes the sphincter (10).

FARADIC-STIMULATION OF INTESTINE. *Small intestine.* Continuing these experiments with other parts of the intestinal tube, we have found

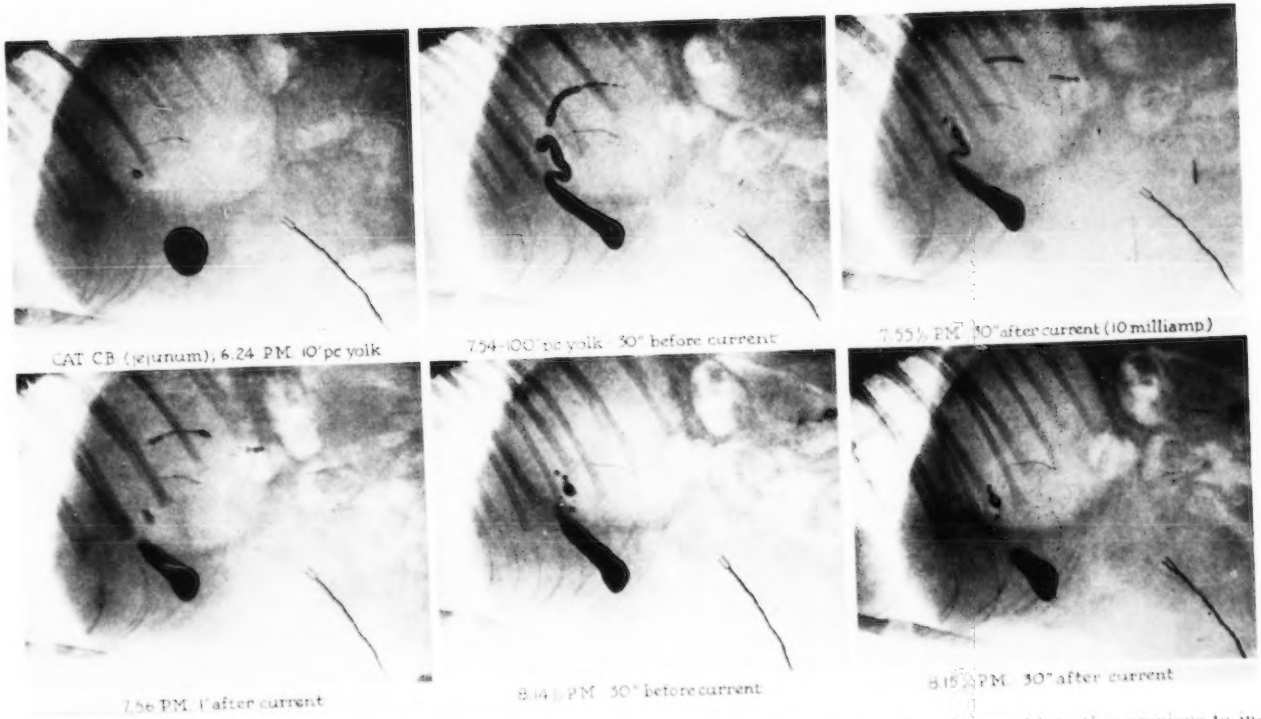

Plate 7. Reaction of gall bladder which is emptying, to faradic stimulation of jejunum. Stimulation of intestine previous to ingestion of food produced slight relaxation of gall bladder.

that stimulation of the small intestine likewise inhibits the emptying of the gall bladder. This has been verified for portions of the duodenum, both cephalad and caudad to the outlet of the bile duct, and for the jejunum (plate 7). The ileum has not yet been tested. Up to this time no conclusive evidence has been found that stimulation of any part of the intestine causes reflex contraction of the gall bladder during fasting. In the series shown in plate 7, stimulation of the intestine before food was given, produced very slight tonus changes in the direction of relaxation.[2]

Cecum. Similarly, it was found that spastic contraction of the cecum induced by application of a tetanizing current, sets up reflexes which cause relaxation of the gall bladder when it is under tonus either during fasting (e.g., *7:32½ p.m.* plate 8) or when it is emptying after a meal (*8:00* and *8:19 p.m.*, plate 8). In one cat only, and this occurred only once in that animal, contraction of the cecum was followed by a delayed contraction of the gall bladder. Especially interesting, in this series are the pictures taken between *8:19* and *8:22 p.m.* inclusive. Here one may see how great is the difference between the contracted and relaxed state of the gall bladder, even when it is nearly empty—a phenomenon that is not appreciated by many investigators who have worked upon the physiology of this organ.

Curiously enough, of all the areas selected, the cecum was found to be the most sensitive, since induced contraction of this appendage caused more pain than that of any other segment of the gut tract. This is especially significant in view of the close relation which clinicians have always felt existed between appendicitis and gall bladder disease. To be sure, in these experiments the inhibitory reflexes set up by faradic stimulation were quickly overcome, since the induction shocks are of short duration; quite different, indeed, from what might happen if there were a stream of impulses arising from chronic irritation of the appendix.

Discussion. The immediate reaction of the gall bladder to stimulation of the visceral musculature, as recorded in these experiments, is apparently explainable only on the basis of a reflex pathway extending from the gastrointestinal tract to the biliary vesicle. It is not intended, however, that evidence presented in this article should be construed as excluding the hormone theory of evacuation. For on the basis of experimental studies made in 1926 (11) in which it was shown that contraction of the gall bladder in cats could be induced by such varied forms of stimulation as intravenous injection of adrenalin and smooth muscle drugs, shaving the cats' legs either before or after the suprarenal glands had been removed,

[2] At the time of operation it was sometimes noted that the current not only produced local ring contraction of the gut but set up shallow peristaltic waves above or below the point of excitation. Apparently these never caused contraction of the gall bladder. But we have no evidence that a "peristaltic rush" or continuous intestinal motility would not affect the gall bladder.

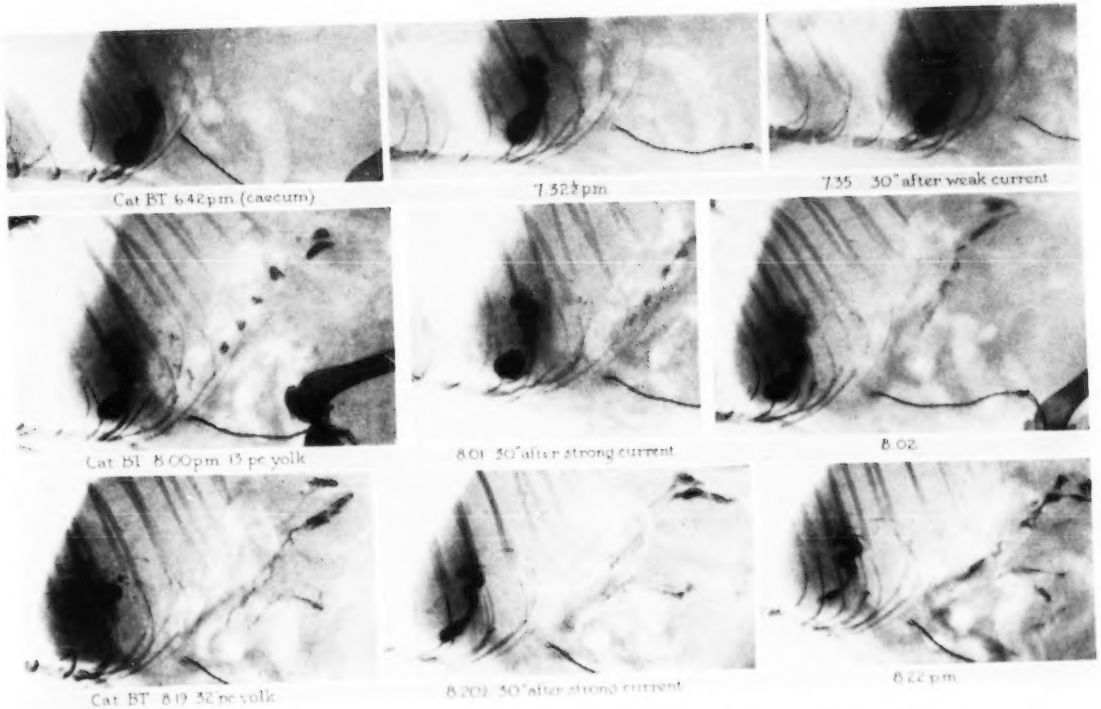

Plate 8. Reaction of gall bladder which is emptying, to faradic stimulation of cecum

and transfusion of blood from one animal to another (or from left to right legs of the same animal)—it was concluded that the gall bladder was subject both to nerve control and to substances in the circulating blood. And in view of Ivy's recent experiments with extracts from the intestinal mucosa (12), it seems conclusive that substances in the circulating blood derived from ingested food also activate the gall bladder and cause its evacuation. To be sure, the relative importance or specific rôle of these different factors, has yet to be worked out; as indeed, has the exact course of the reflex pathway. Nor is it yet clear whether substances in the circulating blood activate the musculature directly or the nervous elements in the gall bladder wall. Halpert's negative findings, when cholecystokinin was added to isolated gall bladders immersed in Locke's solution, would tend to support the latter view (4). At the very least, however, it can be safely said that the gall bladder is under both reflex and hormonal control.

SUMMARY

1. Faradic stimulation of the pars pylorica of the stomach induces sudden contraction of the relaxed gall bladder and ejection of bile into the cystic duct.

2. Hunger contractions of the stomach occur synchronously with rhythmic contractions of the gall bladder and probably account for the periodic emptying of the gall bladder during fasting.

3. When the gall bladder is emptying, after a meal of egg-yolk, faradic stimulation of the stomach, pylorus, small intestine or cecum, temporarily inhibits the emptying of the gall bladder.

4. These observations confirm the existence of a reflex pathway extending from the splanchnic area to the gall bladder.

5. It is suggested that dysfunction and stasis of the gall bladder (with the consequent formation of gall stones) may be due in part to inhibitory reflexes arising from chronically diseased portions of the gastro-intestinal tract.

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THE PHYSIOLOGIC ACTION OF RATTLESNAKE VENOM (CROTALIN)

I. Effect on Blood Pressure: Symptoms and Post-Mortem Observations

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This is the first of a series of papers on the physiologic action of venom from the rattlesnakes *Crotalus horridus* and *Crotalus atrox*. Only a few workers have dealt with this subject from a physiologic point of view. In this respect the most outstanding contributions have been made by Mitchell (1861–1890), by Mitchell and Reichert (1886) and by Rogers (1905). In spite of the splendid work of these investigators many phases of the problem have not, in the light of present-day knowledge, received a satisfactory solution.

Although it has been demonstrated by these various observers that the venom of the rattlesnake, when injected into animals, produces a profound fall in blood pressure, the mechanism by which this is accomplished has not been elucidated. We have, for a number of reasons, undertaken a thorough study of the effect of crotalin on blood pressure and the mechanism involved. We have also observed the symptoms produced and the pathologic changes resulting from injections of crotalin into dogs, rabbits and guinea pigs.

Experimental methods and material. Since, in this research, we made use of the standard physiologic methods, modifying them slightly for our purpose, when necessary, it will not be necessary to give the details of these methods in full. For the most part, the venom which was used in these experiments was obtained from the timber rattler, *Crotalus horridus*, a species which is common in southern and southeastern Minnesota. At first, the snakes were kept on ice overnight, to guard against any possibility of our being bitten during the process of obtaining the venom, and after we became more familiar with their habits, and accustomed to handling them, we were able to secure the venom without subjecting them to refrigeration. We have not experienced any difficulty in keeping them alive. Since they refuse to eat in captivity, each time after taking their venom, which was

about once a month, large pieces of horseflesh were forced down their gullets. A large dish of water was kept constantly before them, from which they occasionally drank, and into which they sometimes crawled and bathed. In the collection of the venom, we have followed the technic of do Amaral (1928) and other workers. When collected, the venom was rapidly measured by aspirating it into a tuberculin syringe, and then diluted with 50 per cent glycerine in Ringer-Locke's solution. When not in use, it was kept in the ice box.[1]

The potency of different samples of venom was reasonably constant, the extreme variation being, perhaps, 100 per cent depending on the condition of the snakes. Usually two snakes would supply us with 30 cc. of 2 per cent venom, which would serve us for about a month. There was no definite evidence that the venom deteriorated in strength during this period. The experiments reported in this paper were completed with two batches of venom.

The venom was, in almost every instance, injected intravenously, to offset variations in the degree of absorption, which are probably great in the case of crotalin, on account of the marked local reaction which this substance induces when given subcutaneously. It is necessary to state that the response to different samples of venom was about as constant as would occur following administration of such drugs as histamine, epinephrine and pituitrin.

EXPERIMENTAL RESULTS IN RELATION TO BLOOD PRESSURE. Early in our experiments, it was discovered that the blood pressure of animals under local or general anesthesia fell precipitately after small doses of venom had been given intravenously. The most characteristic and striking effect of crotalin is seen when a relatively small dose is given intravenously to a mammal. Rabbits often succumb suddenly and dogs may die within two minutes after injection. Thus, when a dose of 0.4 cc. of 2 per cent crotalin is given to a dog (it usually requires, at least, double that amount to produce the same result in a rabbit), the blood pressure shows a precipitous fall after a latent period of from fifteen to thirty seconds. The level to which it falls is within limits roughly proportional to the amount of venom given. A dose of 0.07 cc. of 2 per cent crotalin causes a decided fall, but such small doses are followed by spontaneous recovery to nearly the normal level. Larger doses cause a drop to low levels, with little tendency toward recovery (fig. 1). The speedy death of the animal usually results from doses of this size. In dogs, with one exception, regardless of the size of the doses used, the blood pressure never descended immediately to zero; but a low level of from 30 to 50 mm. of mercury was reached, and this was

[1] The *Crotalus atrox* venom used in these experiments was prepared from a gram of dried venom donated to us by the Antivenine Institute of America through the kindness of Mr. R. H. Hutchison.

maintained for two minutes to an hour or more before gradually reaching zero. After an initial fall to 30 or 40 mm. of mercury subsequent larger doses failed to produce any immediate response. When a lethal dose was given, there followed an initial maximal response, which reduced the blood pressure to shock level. After that it was only a matter of time until zero was reached. Large doses of histamine (1 to 2 mgm.) failed to produce any depressor action after the crotalin had taken effect.

It became apparent, at this stage of the research, that following intravenous injection of crotalin death results from an extreme and characteristic drop in the blood pressure. When the blood pressure was restored, the animal did not die; when the restorative measures were ineffectual in raising the blood pressure the animal died; the animal's chances of recovery were uncertain in proportion to the fall in blood pressure.

As is the case with animals that have been brought into a condition of surgical shock, the administration of fluid in the form of physiologic solution of sodium chloride has but a temporary effect on the blood pressure of the animal. The administration of hypertonic solution of glucose is considerably more effective in restoring blood pressure (figs. 2 and 7). When the animal has received enough venom to cause marked reduction of blood pressure, the hypertonic solution of glucose is inefficacious in producing recovery, and when recovery does occur, it is but transient in nature. Of greater value than hypertonic solution of glucose for the restoration of blood pressure is a solution of gum acacia, 5 per cent, in physiologic solution of sodium chloride and, perhaps even better, a 7 per cent solution of gelatine. In other words, the condition of crotalin shock[2] is closely analogous to that of surgical shock in the response of blood pressure to intravenous administration of fluid. In a badly crotalin-shocked rabbit, weighing 1.5 kgm., we have given as much as 300 cc. of hypertonic solution of glucose, without much benefit to the blood pressure; at necropsy the fluid was found in the retroperitoneal fossae and in the peritoneal cavity.

When the blood pressure is low, following injection of venom, a subsequent injection of even a much larger dose of venom is without effect on the blood pressure. In a dog, in which the injection of 12 mgm. of venom intravenously reduced the blood pressure to a low level, the subsequent administration of 25 mgm. of venom was without effect on the blood pressure (fig. 1). Fifty milligrams of venom produced a slight fall and 120 mgm. produced a further slight fall. This refractory condition of a crotalin-shocked animal has probably a twofold mechanism. When the blood pressure is sufficiently low, the changes induced by crotalin are probably maximal, and any further dosage with this substance would not be expected to result in marked change in blood pressure. In addition, how-

[2] This term is a convenient one for expressing in brief the pronounced reaction of animals to crotalin.

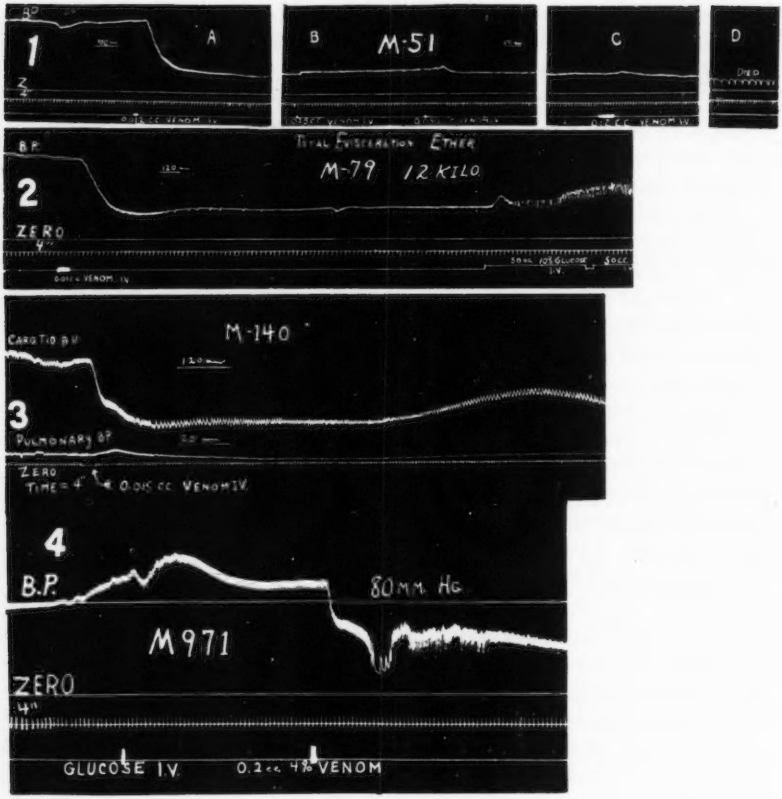

Fig. 1. The dog weighed 18.5 kgm. The effect of an average fatal dose of venom in an etherized dog. The administration of larger doses of venom has no further immediate effect on the blood pressure. The dosage is expressed in terms of pure venom. The interval between *a* and *b* is seven minutes; between *b* and *c* is nine minutes, and between *c* and *d* is ten minutes.

Fig. 2. The dog weighed 12 kgm. The depressor activity of crotalin is augmented in an animal that has been completely eviscerated. All the abdominal viscera were removed except the suprarenal glands. "Shock" is not present in such a preparation. The blood pressure in such preparations is supernormal, probably as a consequence of the ligation of such a large number of blood vessels. The dosage of venom is expressed as pure venom.

Fig. 3. The dog weighed 12 kgm. The increase in pulmonary blood pressure following a dose of venom. The zero is adjusted for pressure in the pulmonary artery. Spontaneous recovery of the carotid blood pressure is evident. The dosage of venom is expressed in terms of pure venom.

Fig. 4. The effect on the blood pressure of 0.2 cc. of 4 per cent venom given intravenously to a decapitated dog. The usual depressor action of crotalin resulted.

ever, there is a certain amount of desensitization following the first injection. In a dog that previously had received an injection of 20 mgm. of venom, the blood pressure had returned spontaneously to a physiologic level in the course of about ten minutes. A subsequent administration of larger doses produced only a slight drop in the blood pressure. The temporary condition of desensitization is soon gone, so that in twenty-four hours the same dose of venom produces practically the same fall in blood pressure.

THE MECHANISM OF THE DEPRESSOR ACTION OF CROTALIN. Statements can be seen in the literature to the effect that rattlesnake venom kills as a result of universal muscular paralysis and that the action on the neuromuscular apparatus is somewhat similar to that of curare. We do not consider this to be well founded, since we have found artificial respiration to have no influence on the toxicity of this poison, and the administration of crotalin to a completely curarized dog produced the same fall in blood pressure. The injection of crotalin into a decapitated preparation, is still followed by the usual fall in blood pressure. In this preparation, on account of the initial low blood pressure, consequent on the ablation of the vasomotor centers, the action of crotalin is not so dramatic (fig. 4). However, there is little reason for supposing that the fall in blood pressure has an essentially central origin. Histamine, which is recognized as peripheral in its activities, shows the same behavior on blood pressure in the decapitated dog. The evidence that the fall in blood pressure from crotalin is not cardiac in origin is that the administration of hypertonic and viscous fluid is decidedly beneficial to a crotalin-shocked animal, which would not be the case if the fall in blood pressure were of cardiac origin.

In view of the fact that the venom of certain snakes is known to contain a strong fibrin ferment, we considered the possibility that the fall in blood pressure was due to intravascular thrombosis. That this is not the case was definitely proved by repeatedly bleeding a dog, defibrinating the blood by whipping, and reinjecting this until no more fibrin was obtained. The administration of 0.3 cc. of 2 per cent crotalin into this animal gave the usual result. Following this experiment, we studied the action of crotalin on a heparinized dog; 350 mgm. of heparin was injected intravenously into an animal weighing 7 kgm. After the injection of the usual amount of crotalin, the typical fall in blood pressure resulted. These observations convinced us that crotalin did not depend on intravascular thrombosis for its effect on the blood pressure, a view which was substantiated by necropsy, since the blood of animals which had died from crotalin usually was not coagulable.

To eliminate the influence that the vagus nerve might have on the blood pressure following the injection of crotalin, a dog weighing 2 kgm. was given 7 mgm. of atropine intravenously. About six minutes later, the right

vagus nerve was stimulated in the neck. A slight rise in blood pressure followed. After about eight minutes more had elapsed, the usual dose of crotalin was given intravenously, and the same depressor action followed as in a dog not treated with atropine. The experiment was repeated on an animal that had received enough ergotoxin to minimize the pressor action of small doses of epinephrine. The usual fall in blood pressure was obtained.

The subcutaneous injection of about seven times the dose given intravenously reduced the blood pressure to 60 mm. of mercury within forty minutes. Since an animal in nature receives the poison in this manner the end results are the same; the only difference is one of time, as might be expected.

Since the venom manifests its usual effect in the decapitated organism, the fall in blood pressure obviously is not central, and therefore must be either cardiac or peripheral in nature. The fact that the action of venom is accompanied by tachycardia may be offered as evidence for the peripheral nature of its action, in accordance with the familiar law of Marey. To establish this matter definitely, a heart-lung system was set up according to the method of Starling. The peripheral resistance was kept constant at 105 mm. of mercury. The addition of 8 mgm. of venom to the reservoir containing 600 cc. of blood was without any influence on the cardiac output. The heart ejected its usual quota over a period of fifteen minutes, when the observation was discontinued. By elimination, therefore, the fall in blood pressure resulting from the venom is peripheral in origin.

Waud (1927) found that in histamine shock, in peptone shock, and in anaphylactic shock, the blood showed a marked, although fleeting, reduction in viscosity, to which he attributed the fall in blood pressure. There is no reduction in viscosity of the blood following administration of crotalin. There is, on the contrary, a marked increase in viscosity according to Baldes.

In recent years, the attempt has been made to implicate the liver in peptone shock and in anaphylactic shock. To eliminate the influence of the liver and other viscera we have, therefore, repeated our experiments on dehepatized and on eviscerated preparations. The hepatectomy was performed by Mann's (1924) three-stage operation, or by the two-stage technic of Markowitz and Soskin (1927–1928). Complete abdominal evisceration was performed by the usual technic employed in this laboratory (Mann, 1925). Invariably, the administration of venom to a liverless or to a completely eviscerated dog brought about the usual fall in blood pressure (fig. 2). The fall in blood pressure is, therefore, not dependent on spasm of the hepatic veins or on congestion in the splanchnic viscera, as has been supposed by some to be the case in anaphylactic shock.

We considered the possibility that the fall in blood pressure might be due to pulmonary constriction, with the result that the blood did not

get to the left auricle. Accordingly, under artificial respiration a cannula was placed in the left branch of the pulmonary artery at the hilum of the lung. Following intravenous injections of crotalin there was invariably a slight rise in pulmonary pressure, followed by a fall which was probably dependent on a deficient return flow of blood to the right side of the heart (fig. 3).

In one experiment, a cannula was placed in the thoracic duct of a dog fed on fat, and the flow of lymph was recorded by making an electric contact through a signal magnet for every drop that exuded from the cannula. The flow of lymph was approximately doubled immediately following administration of crotalin, and the lymph took on a distinctly reddish color. Within about a minute after the injection, the flow had returned to a slightly greater rate than normal.

PLETHYSMOGRAPHY OF THE VARIOUS ORGANS. Accompanying the depressor action of crotalin, there is invariably an increase in volume in the hind limb (fig. 5). We might state that the increase is probably much greater than that represented in the tracing, since we omitted the precaution of filling the plethysmograph with warm water. This peripheral vasodilatation is probably general; thus, Mason, who has worked out a technic for plethysmography of the thyroid gland in this institution, found that the gland enlarges immediately following injection of crotalin. In the spleen there is invariably pronounced decrease in volume accompanying the immediate fall in blood pressure (fig. 6). This is also true of the kidney (fig. 7), the liver and the intestine. The shrinkage of the splanchnic viscera roughly parallels the fall in blood pressure. The decrease in volume usually varies in duration from about five to ten minutes, after which extreme expansion occurs, so that in the spleen, kidney and intestine, frequent adjustments of the tambour or the piston recorder become necessary in order to keep the writing point on the drum. In the spleen, the initial marked decrease in volume which parallels the fall in blood pressure was often interrupted by temporary expansion, only to be followed again by shrinkage. We have seen this phenomenon, also, in the kidney, and more frequently in the intestine. However, invariably, as the blood pressure fell rapidly, the splanchnic viscera showed universal marked decrease in volume. Within a variable length of time, in some cases indeed within a few minutes, after the initial vasoconstriction the splanchnic viscera all increased profoundly in volume.

In regard to the mechanism of the fall in blood pressure, plethysmography, therefore, has apparently supplied a definite answer. It appears that enough blood is diverted to a widely opened vascular system in the musculature of the animal, and the consequent alteration in the capacity of the circulation is responsible for the immediate fall in blood pressure.

On several occasions, we have studied the action of crotalin on the vol-

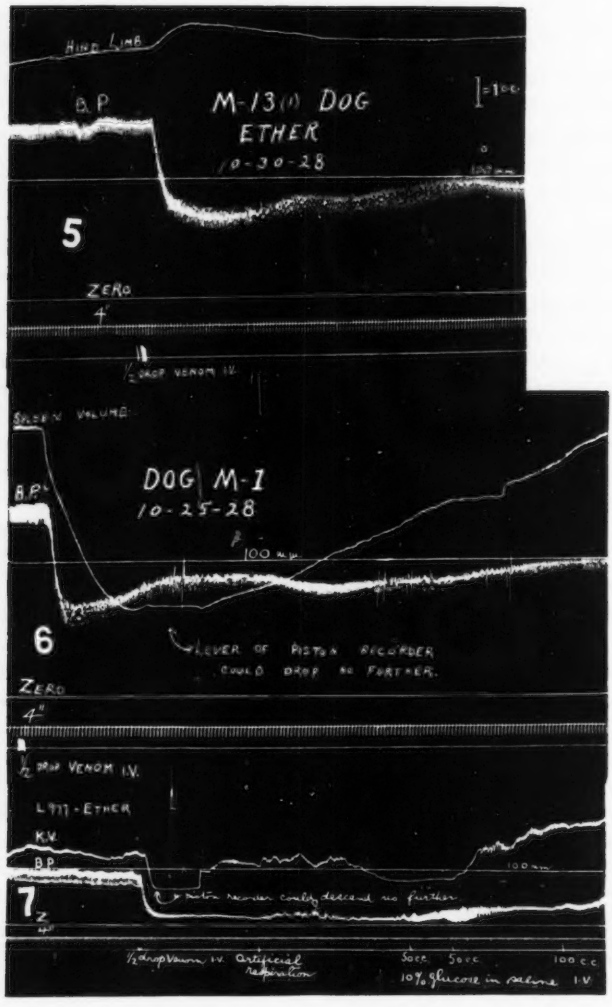

Fig. 5. The dog weighed 10 kgm. The expansion of the hind limb invariably accompanies the immediate depressor action of crotalin. The dosage of venom is expressed as pure venom.

Fig. 6. The dog weighed 14 kgm. The marked shrinkage of the spleen invariably accompanies the immediate action of crotalin. The capacity piston of the recorder was 15 cc. The dosage of venom is expressed as pure venom.

Fig. 7. The dog weighed 19 kgm. The effect of venom on renal volume. There was marked shrinkage in the volume of this organ followed by a quiek return to its normal size. Improvement in blood pressure was effected by solution of glucose. The animal almost certainly would have died in the absence of this treatment. The dosage of venom is expressed as pure venom. The capacity of piston recorder was 15 cc.

ume of a viscus in the intact animal. Following the method of Hargis and Mann (1925) we have made observations regarding the influence of crotalin on the blood pressure of an animal in which a renal plethysmograph had been applied several days previously. The results were identical with what is observed under general anesthesia. The recovery from low blood pressure was definitely facilitated by the absence of general anesthesia.

SYMPTOMS AND POST-MORTEM OBSERVATIONS. The symptoms following intravenous injections of crotalin into dogs may be briefly stated as repeated vomiting, defecation, urination and marked collapse.

The following observations indicate a typical post-mortem picture in a dog that had succumbed to crotalin. There was marked rigor mortis. Numerous petechial hemorrhages were found on the upper and lower eyelids; these resulted in an intensely inflamed appearance. Likewise, petechial hemorrhages were to be observed in the tongue, especially about the periphery. In the thorax, the contents of the anterior mediastinum were markedly infiltrated with these hemorrhages and they were found in the adventitia of the ascending aorta. The right auricle manifested a large subpericardial hemorrhage, which measured 2 by 1.5 cm. Hemorrhages were present also on the surfaces of both ventricles, over the central tendon of the diaphragm, under the serous surface of the gall bladder, and over all the peritoneal organs, especially the greater omentum. The serous surface of the entire alimentary tract below the esophagus was dotted with hemorrhagic areas which were most marked in the descending colon. The pancreas was the site of tremendous hemorrhagic infiltration. The liver was mottled and purplish and on section was extremely congested. The spleen was slaty-gray, and congested on section. The kidney was grayish externally. On section reddish streaks were seen in the medulla. There was a submucous hemorrhage in the renal pelvis. The suprarenal glands apparently were normal externally, but on section punctate hemorrhages were found, especially marked in the medulla, which in consequence was brown. The bile was brown, apparently from blood. The mesenteric lymph nodes were hemorrhagic. In the interior of the stomach, small intestine, and colon there were large areas of hemorrhage, and the small intestine was full of comparatively fresh blood. There were multiple diffuse and punctate hemorrhages in the brain, especially in the corpus striatum. The blood in the heart and great veins was not clotted.

These changes represent the typical end results of acute crotalin poisoning in the dog. It should be stated, also, that the urine was frequently tinted, and in one case contained enough blood to make it a brilliant red.

The changes in the rabbit are in the main identical with these.

In the guinea pig the symptoms differed from those in the dog, as did also appearances at post-mortem. When a guinea pig receives an injection of crotalin, the animal scurries about as though very much frightened. It

soon becomes dyspneic, and wheezes and râles become audible to the unaided ear. Its coat roughens. Within a few minutes, it becomes comatose and dies. At necropsy, the most marked feature noted in the guinea pig was the emphysema of the lungs; in some cases this emphysema was comparable to that produced by histamine or by anaphylaxis.

The microscopic anatomy involved in the changes which have been described will be reported in another paper. It is pertinent to describe here the histologic appearance of a portion of the greater omentum obtained from the dog concerned in the necropsy mentioned. This tissue when stained with eosin and cleared in xylol, presented an excellent picture of the hemorrhages when viewed with a low-power binocular microscope. It was observed that the hemorrhages occurred, almost without exception, in the smaller veins. Veins that measured 0.5 mm. in diameter were ruptured and many of the veins in communication with a vessel of this size showed definite breaks through which the blood had been poured into the surrounding tissues (fig. 8).

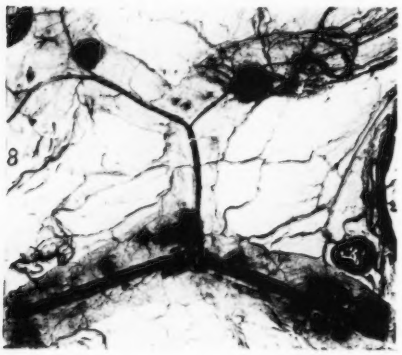

Fig. 8. Stained and cleared portion of the greater omentum taken from a dog that died from an injection of venom. Congestion and hemorrhage on the venous side are evident (× 5).

Comment. The action of rattlesnake venom is different from the action of any other venoms, reports of which we have encountered in the literature. Gunn (1912) found that cobra venom had an epinephrine-like action, although later he indicated that it also possessed a digitalis-like action. On perfusion through the blood vessels of a frog, cobra venom caused constriction of the arteries. In the mammal, it caused acceleration and augmentation of the isolated heart, and it induced a preliminary fall in blood pressure due to paralysis of the vasomotor center. This fall in blood pressure was followed by a supra-normal rise in blood pressure due to constriction in the arterioles. On these grounds, Gunn considered cobra venom to be more analogous to epinephrine than to strophanthin to which it had been likened by Elliot. In vitro, the analogy to epinephrine was very close. The venom causes contraction of some kinds of unstriated muscle and relaxation of others, and Gunn, therefore, reasoned that it probably acts on the sympathetic nerves. When cobra venom was given in small doses gradually, the preliminary fall in blood pressure, which occurred only with large

doses, did not occur, but, from the first, there was produced a steady rise in blood pressure which often attained high levels.

It is remarkable that the results following the intravenous injection of crotalin should bear such a striking similarity to anaphylactic shock; this resemblance automatically brings these symptoms into relationship again with peptone shock, with histamine shock, and with the results following the administration of various animal and bacterial extracts. Biedl and Kraus (1909) were the first to show that the characteristic alteration in the circulation in anaphylactic shock was a sudden, profound fall in blood pressure. Pearce and Eisenbrey (1910) reported the same change, and an analysis of their tracings reveals an instructive similarity between crotalin shock and anaphylactic shock, as concerns the reaction of the circulation.

SUMMARY

There is presented in this paper a description of the effect of intravenous injection of small doses of rattlesnake venom on the blood pressure of dogs, rabbits and guinea pigs. In each case, there is a remarkably sharp, profound drop in blood pressure to a level characteristic of shock. An analysis of the mechanism of this fall in blood pressure has shown that it is not central, since it occurs in the decapitated preparation, that it is not cardiac, since an equivalent amount of venom does not impair the ability of the heart to expel blood in a heart-lung perfusion against a resistance of 105 mm. of mercury. The fall in blood pressure is not due to intravascular thrombosis, and by elimination, therefore, is peripheral in nature. It still occurs in the dog that is dehepatized or eviscerated. The exact mechanism of this fall in blood pressure has not been elucidated. Plethysmography of the splanchnic viscera (liver, spleen, kidney, and intestine) shows that accompanying the fall in blood pressure there is a decided decrease in volume of these organs, and that after a variable lapse of time they expand markedly, which is in agreement with the post-mortem appearance of extreme congestion and hemorrhage in these organs. The hind limb invariably is markedly expanded during the active fall in blood pressure. This property of rattlesnake venom in inducing a profound fall in blood pressure is strikingly similar to the results following injection of a suitable antigen for a sensitized dog.

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THE PHYSIOLOGIC ACTION OF RATTLESNAKE VENOM (CROTALIN)

II. The Effect of Crotalin on Surviving Organs

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In the course of our studies on the physiologic properties of crotalin, we had occasion to perfuse the isolated heart, both by the technic of Locke and Rosenheim (1907–1908) and by the method of Starling (1912), the so-called heart-lung perfusion. Two striking observations resulted from these experiments: 1, the contractile power of the rabbit's heart was quickly abolished when enough venom was added to the perfusing fluid, and 2, the permeability of the capillaries of the lung was markedly increased for fluid when venom was added to the defibrinated blood in the reservoir, so that the lungs became intensely edematous in about fifteen minutes. On the basis of these experiments, we investigated the effects of crotalin on the fatigue curve of the gastrocnemius muscle of the frog, on the response of the perfused uterus of the virgin guinea pig and on the perfused intestine of the rat. The results thus obtained form the basis of this communication.

The effect of crotalin on the perfused heart of the rabbit. A rabbit was etherized and exsanguinated by bleeding from the abdominal aorta and inferior vena cava. The thorax was opened and the heart carefully excised, care being taken to leave a long strip of aorta. The heart was gently placed in cold Ringer-Locke's solution. The chambers of the heart were irrigated and perfused for several minutes with cold Ringer-Locke's solution. When it appeared that the heart was quite bloodless, it was suitably trimmed, and tied to the cannula of the perfusing apparatus of Locke and Rosenheim. This was slightly modified to permit the taking of graphic tracings. When the heart had settled down to a vigorous and regular beat, venom was added to the perfusing fluid in the proportion of 1 mgm. to 100 cc. Within five minutes, the beat had become much feebler, and within eight minutes, the beat had stopped completely and the heart was in an extremely edematous condition (fig. 1). This result was invariable. The addition of a minute trace of venom to the perfusing fluid resulted in two striking changes: the beat immediately weakened and finally stopped, and the heart became edematous. By using smaller con-

centrations of venom, we have been able to render the heart so intensely edematous as to make it seem possible that the cessation of the heart beat depended on the extreme edema. In concentrations of 0.75 mgm. of crotalin for each 300 cc. of perfusing fluid, the effect of the venom was more manifest in causing edema than in disabling the musculature of the heart (fig. 2).

This experiment indicates that the venom can act in the absence of blood. In fact, the suggestion is unavoidable that it acts much more injuriously on the muscle of the heart in the absence of blood, since rabbits are often able to withstand a concentration of venom that would disable an isolated heart very quickly. The experiment suggests that one action of venom, which may be responsible for its effect on blood pressure, and the final occurrence of extreme edema of the viscera, is that of an endothelial poison.

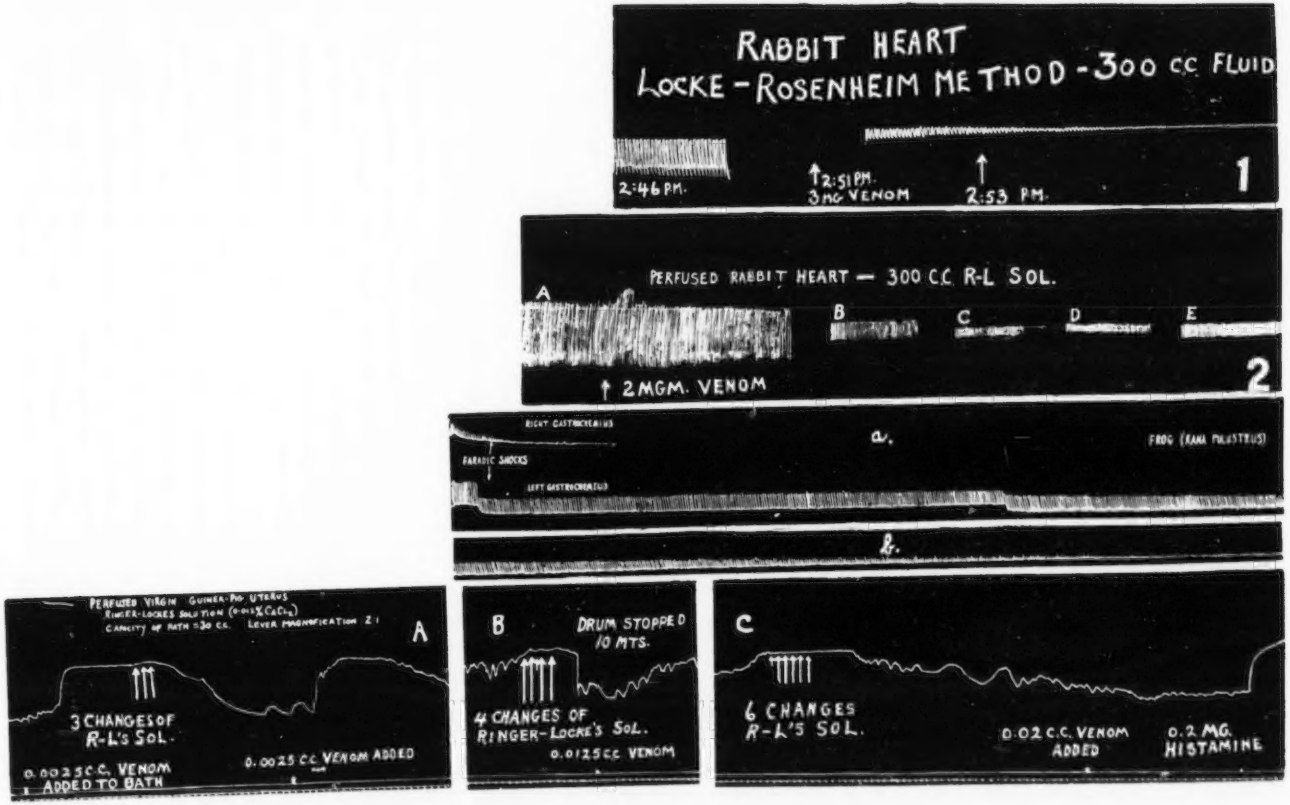
THE EFFECT OF CROTALIN ON HEART-LUNG PREPARATION. About 300 cc. of blood were obtained from a large etherized dog. The blood was defibrinated and filtered. This blood was added to the reservoir of a heart-lung apparatus. A dog weighing 10 kgm. was then etherized, and a heart-lung preparation was arranged according to the well known method of Starling. The level of the reservoir was adjusted so that the cardiac output was about 250 cc. each minute, at a peripheral resistance of about 105 mm. of mercury. The total quantity of blood in the reservoir was estimated at about 600 cc. Electrocardiographic tracings of the heart were taken, the leads being at the base of the heart and the superior surface of the liver, where it presses against the diaphragm. The addition of 0.4 cc. of 2 per cent venom induced only a slight change in the contractile power of the heart. The flow of blood into the reservoir was not obviously altered, indicating that the cardiac output was not impaired, in spite of the fact that the peripheral resistance was kept fixed at 105 mm. of mercury. Since this is the dose of venom that usually is employed to produce crotalin shock in a dog weighing 10 kgm., containing presumably the same amount of blood, the conclusion is inevitable that, when such a dog is given this dose of venom, the resultant fall in blood pressure is not of cardiac origin. At the end of fifteen minutes it was noted that the lungs had a rubbery consistence, and that froth was issuing from the trachea; these observations were confirmed by the marked diminution of blood in the reservoir. This condition progressed so that at the end of about twenty minutes the lungs were

Fig. 1. The reaction of perfused rabbit heart to 3 mgm. of crotalin.

Fig. 2. The reaction of perfused rabbit heart to 2 mgm. of crotalin.

Fig. 3, *a* and *b*. Effect of venom on the right gastrocnemius muscle of a frog as compared with its fellow which was removed before the injection of the venom.

Fig. 4. The effect of repeated doses of venom on the perfused uterus of the guinea pig. Interval between *A* and *B*, 15 minutes; between *B* and *C*, 16 minutes.


RABBIT HEART
LOCKE-ROSENHEIM METHOD - 300 CC FLUID
2:46 P.M.
2:51 P.M.
3MG VENOM
2:53 P.M.
1
PERFUSED RABBIT HEART — 300 CC R-L SOL.
A
B
C
D
E
2MGM. VENOM
2
RIGHT GASTROCNEMIUS
FARADIC SHOCKS
LEFT GASTROCNEMIUS
a.
FROG (RANA PALUSTRIS)
b.
PERFUSED VIRGIN GUINEA-PIG UTERUS
RINGER-LOCKES SOLUTION (0.01% CaCl2)
CAPACITY OF BATH = 30 CC. LEVER MAGNIFICATION 2:1
A
3 CHANGES OF R-L's SOL.
0.0025 C.C. VENOM ADDED TO BATH
0.0025 CC VENOM ADDED
B
DRUM STOPPED 10 MTS.
4 CHANGES OF RINGER-LOCKE'S SOL.
0.0125 CC VENOM
C
6 CHANGES R-L'S SOL.
0.02 C.C. VENOM ADDED
0.2 MG. HISTAMINE

Figs. 1–4

solid, and the experiment had to be terminated because the blood had practically disappeared from the reservoir.

We have observed a considerable number of heart-lung perfusions, and we have never seen edema of the lungs occur so rapidly under the usual experimental conditions which accompany a heart-lung perfusion. We have repeated this experiment several times, and believe, therefore, that this result is genuine, and that the experiment cannot justly be criticized on the grounds that perfused organs are notoriously prone to the development of edema. The same might be said of the edematous condition of the heart of the rabbit that is being perfused with Ringer-Locke's solution containing a trace of crotalin. The perfused heart in our experience beats at least six hours before there is any marked edema, and often for eight hours, with only slight edema. The observations in the foregoing experiments, therefore, confirm each other as regards the influence that crotalin has on the increased permeability of capillaries for fluid.

On two occasions we removed the hearts of rabbits killed with crotalin, and perfused in the manner already described. The hearts were in good condition for about four hours, at the end of which time they were edematous, and pulsated only feebly.

In view of the fact that crotalin was poisonous in this series of experiments in virtue of its action on cardiac muscle, and its apparently poisonous action on endothelium, the next two experiments were performed.

FATIGUE CURVE OF THE GASTROCNEMIUS MUSCLE OF A FROG. The gastrocnemius muscle of a decerebrate frog was excised, care being taken to avoid hemorrhage, and a large dose (10 mgm.) of venom was injected into the dorsal lymph sac. Enormous doses of crotalin are required to affect the frog. A dose of venom that will kill an average sized dog may occasionally inconvenience a frog weighing 30 grams, although the giving of enough venom is followed by a lethal result. A fatigue curve was constructed for the excised gastrocnemius muscle by recording the isotonic contractions resulting from make-and-break faradic shocks that were applied to the muscle every second. The gastrocnemius muscle of the other leg was then removed, about half an hour having elapsed since the injection of venom. The fatigue curve of this muscle was definitely impaired, as compared with its fellow. To make such an experiment quantitative is difficult, but we varied the conditions in many ways, always, however, with the same result: the muscle of the venomized frog had much feebler contractile power (figs. 3 *a* and *b*). In one experiment, the right leg of a frog was ligated above the knee, so as to stop the blood supply to the corresponding gastrocnemius muscle. The frog was then given a large dose of venom into the dorsal lymph sac. About half an hour after injection of the venom, the gastrocnemius muscle of the left leg was excised, and a fatigue curve was constructed in the usual manner. When the muscle no longer responded,

the gastrocnemius muscle of the ligated leg was removed and was prepared for stimulation. Here again, the muscle that had been protected from the venom was much more vigorous, and showed a much more prolonged fatigue curve, in spite of the fact that its circulation had been cut off for thirty-five minutes.

THE EFFECT OF VENOM ON THE PERFUSED UTERUS OF THE VIRGIN GUINEA PIG AND INTESTINE OF THE RAT. The technic adopted was the familiar one of Dale (1913). The perfusing fluid was Ringer-Locke's solution, containing half the usual quantity of calcium. The capacity of the bath was 30 cc. The addition of venom regularly produced maximal contraction of the uterus, after a latent period of about thirty seconds. When the venom was removed by washing with fresh Ringer-Locke's solution, the organ relaxed, and the addition of venom was again followed by a contraction which this time was broken by slight, rhythmic, superimposed waves. Since the response to a second addition of venom was not quite so vigorous, we attempted to obtain some evidence that this might have been due to desensitization. Accordingly, the uterus was washed with fresh Ringer-Locke's solution, and venom again was added. There was an increase in tonus which was not so marked as in the previous case. In about half an hour the venom was washed away, and this procedure was followed by relaxation of the uterus. The addition of venom was now ineffectual in causing contraction; however, the addition of histamine promptly resulted in a maximal contraction, which was unbroken by any rhythmic waves (fig. 4). We have repeated this experiment on several occasions, always with the same result. The experiment is instructive for a number of reasons. The long latent period (half a minute) may be contrasted with the almost immediate response when antigen is added to the sensitized uterus of the guinea pig. The progressively weaker response of the uterus to venom appears to indicate that some substance is being expended which acts as an intermediary in the oxytocic action of venom, and this observation may be brought in line with the effect of venom on the blood pressure. It has been pointed out in a former paper that when an animal's blood pressure spontaneously returns to a physiologic level within about half an hour following a sublethal dose of venom, the animal is often refractory to subsequent doses of venom.

A strip of intestine of a rat was perfused according to the well-known method of Magnus. After the addition of 0.2 cc. of 4 per cent crotalin to the 45 cc. of perfusing fluid there followed almost immediately a relaxation of the intestinal strip. The relaxed condition was of short duration as a slow contraction soon brought the recording needle back to the original level. It is of interest to note that the response of the intestine of a rat to crotalin is the opposite of the response of the uterus of the guinea pig.

COMMENT. The foregoing experiments may be used as evidence that crotalin is a violent endothelial poison and that it also has a profound influ-

ence on muscular tissue. Its influence on cardiac and skeletal muscle is to enfeeble the contractile property of the tissue, and it acts as an intense stimulant of the excised uterus of the virgin guinea pig, which action differs from the anaphylactic reaction of this organ in having a longer latent period and in being desensitized with much more difficulty. Crotalin differs from histamine, in that the uterus may fail to respond to crotalin, when it still possesses the capacity to respond to histamine.

Gunn and Heathcote (1921) found that a concentration of 1:5000 of cobra venom stopped the heart in a systolic position in eight minutes. A concentration of 1:2,000,000 stopped the heart in seventy-three minutes. The venom diminished the coronary flow from the beginning, independently of the alteration in the heart-beat.

SUMMARY AND CONCLUSIONS

The influence of a dilute solution of rattlesnake venom was studied on various perfused organs of animals with the following results:

1. When venom is added to the perfusing fluid of a rabbit's heart, perfused with blood-free Ringer-Locke's solution, the contractile power of the heart was rapidly abolished, and the heart became edematous within a period of about twenty minutes or less, depending on the dosage.

2. When venom is added to defibrinated blood, perfused through a heart-lung preparation (Starling), the heart continues to beat against a peripheral resistance of 105 mm. of mercury in spite of the fact that the quantity of venom added was lethal for a dog containing about the same quantity of blood. The lungs, during the experiment, became intensely edematous, so that, at the end of eighteen minutes, the experiment had to be terminated because about 500 cc. of fluid had disappeared into the lung.

3. The gastrocnemius muscle of a frog that is injected with crotalin has a much feebler fatigue curve than its fellow of the opposite side, which has been protected from the venom.

4. The perfused uterus of the virgin guinea pig reacts to crotalin by a maximal contraction. This contraction differs from the anaphylactic response of this organ in two particulars: there is a sufficiently long latent period to suggest intervention of some enzyme mechanism, and desensitization is slight, and requires repeated doses of venom. When the uterus finally fails to react to crotalin, histamine still evokes a maximal response. The perfused intestine of the rat is relaxed when crotalin is added to the perfusing fluid.

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THE PHYSIOLOGIC ACTION OF RATTLESNAKE VENOM (CROTALIN)

III. The Influence of Crotalin on Blood, in Vitro and in Vivo

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In this paper is presented the marked action of crotalin on the volume of erythrocytes, as a result either of the intravenous injection of crotalin, or in consequence of the addition of this venom to heparinized or defibrinated blood in a test-tube. The phenomenon promises to be of material assistance in the quantitative investigation of certain phases of immunity toward crotalin. It also presents in a striking light the ability of crotalin to influence the migration of water through cell membranes.

In the course of our studies on the changes in blood that occur following the injection of crotalin, we were unable to detect any change in the counts of erythrocytes or leukocytes, or in concentration of hemoglobin for approximately five minutes following injection of the poison. Platelet counts taken during this time did not change. (There was no attempt to obtain absolute values. Venous blood was used, and the diluting fluid was 1 per cent sodium citrate.) Apparently, therefore, nothing occurred in the formed elements of the blood which would explain the rapid fall in blood pressure. In order to confirm observations which indicated that the fall in blood pressure was not due to the rapid loss of plasma from the circulation, we centrifuged a specimen of blood before and after administration of the venom, in order to get the ratio of corpuscles to plasma (fig. 1). A paradoxic result was obtained; in spite of the fact that both bloods were treated identically, in the specimen removed immediately, or as long as forty minutes after the injection of the venom, there was a huge increase in the proportion of corpuscles to plasma. This observation was invariable, and puzzled us, in view of the fact that the blood counts did not indicate any such loss of fluid from the circulation. Two possibilities presented themselves as an explanation: 1, following the injection of venom, the viscosity of the blood was so increased as to impede markedly the settling of the corpuscles on centrifugation, and 2, the cell volume was markedly increased following the administration of venom. The former possibility was readily discounted by prolonged high speed centrifugation of the blood,

in which case there was still a decided increase in the ratio of corpuscles to plasma. Accordingly, the corpuscles of the dog were examined microscopically before and after the administration of the venom, and it was clear that there was a marked increase in the volume of the corpuscles following the intravenous injection of crotalin. Whereas, before the injection, the corpuscles showed a tendency to be crenated, immediately after the injection of the venom, the corpuscles were spherical; there was no trace of the central depression when the corpuscles were rotated by pressing on the coverslip. This increase in the size of the erythrocytes was obviously of sufficient magnitude to account for these observations. To obtain more information on this phenomenon, the next experiment was performed. Varying quantities of venom were added to heparinized blood in vitro, and the samples were centrifuged simultaneously. The result invariably was that blood containing venom showed a marked increase in the ratio of corpuscles to plasma. In some cases, the increase was so marked that the centrifuge tube showed merely a thin layer of plasma above the corpuscles, after fifteen minutes' spinning at high speed (fig. 2).

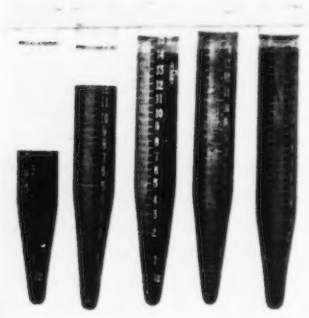

Fig. 1. Tube *1* taken before injection of crotalin. Tube *2* taken one minute after injection of 0.4 cc. of 2 per cent crotalin intravenously into a dog. Tube *3* taken six minutes after injection. Tube *4* taken eleven minutes after injection. Tube *5* taken thirty-six minutes after injection. All tubes were centrifuged for fifteen minutes immediately after blood was removed from the dog.

From these data, it is clear that the change in the ratio of corpuscles to plasma which constantly occurs in the blood of an animal that has received injection of crotalin, is not due to the loss of fluid from the circulation. These observations were invariable until we began making use of oxalated blood. Under these conditions, the addition of venom to blood in the centrifuge tube did not bring about a change, either in cell volume as observed under the microscope, or in the ratio of corpuscles to plasma in the centrifuged specimens. Our previous results had been so striking and so repeatable that we were loath to admit them erroneous. Accordingly, we repeated the former technic in every detail, making use of heparinized or defibrinated blood, and the original observations were confirmed. The inference from these observations was that oxalate prevented the expansion of the erythrocytes. When oxalated blood is recalcified and venom

added, the venom exerts its typical action again, as regards the ratio of corpuscles to plasma. It is barely possible that this observation is de-

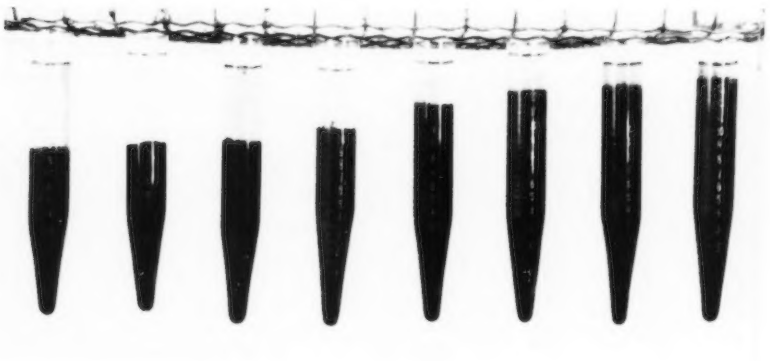

Fig. 2. The effect of adding venom in minute, increasing quantities on the ratio of corpuscles to plasma. Tube *1* is a control, tube *2* had 0.0003 cc. of 2 per cent venom, tube *3* had 0.0006 cc., tube *4* had 0.0009, and so on in series. None of these tubes showed hemolysis or turbidity of the plasma. The tubes were centrifuged immediately after addition of venom.

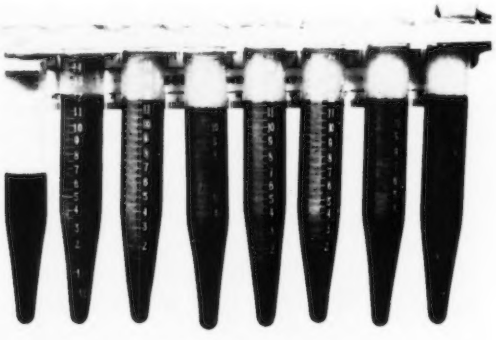

Fig. 3. The blood in this series was treated in the same manner as demonstrated in figure 2, except that the tubes were not centrifuged until thirty minutes after the addition of venom. All of these tubes, except the first, showed hemolysis and progressively increasing turbidity.

pendent, not on the presence of calcium per se, but on the removal of the oxalate ion.

One of the outstanding properties of crotalin is its ability to induce hemolysis of erythrocytes. Our experiments have supplied some interesting data

on the mechanism of this hemolysis. They have shown clearly that hemolysis is secondary to the swelling of the erythrocytes. In a series of eight tubes, the first being used as a control, graduated doses of venom were added, 0.003, 0.006, 0.009 cc. and so on in series. The tubes were centrifuged for fifteen minutes immediately after the addition of the venom. The change in cell volume was roughly proportional to the amount of venom added. The cell volume in the tube containing 15 cc. of normal blood was 8.7 cc.; the remainder of the tubes read as follows: 8.6, 9.8, 10.6, 11.8, 12, 13 and 13.8 cc. (fig. 2). Hemolysis did not take place in any of these tubes. This experiment was repeated with the exception that the tubes were allowed to stand at room temperature for thirty minutes after the addition of the venom. After they were centrifuged for fifteen minutes the cell volume of each of the tubes was between 11 and 12 cc. All except the control showed progressively increasing hemolysis and turbidity (fig. 3). In each of our experiments on dog's blood the expansion of the erythrocytes occurred at once, but hemolysis did not appear until after a latent period of twenty or thirty minutes, unless very large doses of venom were used. In such instances hemolysis occurred in the tubes when they were centrifuged for only ten minutes after the venom was added. This evidence indicates that the hemolysis resulting from crotalin is associated with the swelling of the erthrocytes. It is possible that the venom acts on the capsule of the cell, thus altering its permeability for fluid. Hemolysis occurs when the injury to the capsule is sufficient. When crotalin is added to erythrocytes that have been thoroughly washed with several changes of sodium choloride solution, there is neither an increase in the ratio of corpuscles to supernatant fluid, nor is there any hemolysis, unless normal plasma is added to the mixture (fig. 4).

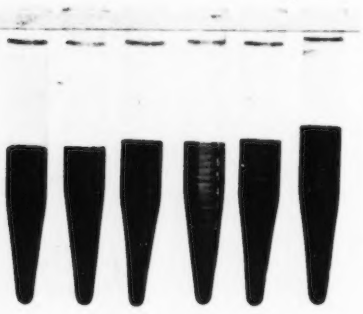

Fig. 4. Tube *1*, normal dog's blood. Tube *2*, normal cells washed with sodium chloride solution. Tube *3*, cells washed with sodium chloride solution and 0.0021 cc. 2 per cent crotalin added. Tube *4*, cells washed with sodium chloride solution and 0.02 cc. 2 per cent crotalin added. Tube *5*, cells washed with sodium chloride solution, 1 drop of plasma and 0.0021 cc. 2 per cent crotalin added. Tube *6*, cells washed with sodium chloride solution, 0.5 cc. of plasma and 0.0021 cc. crotalin added. All tubes were centrifuged fifteen minutes.

COMMENT. The evidence is clear that the mechanism of hemolysis, under the influence of crotalin, is essentially concerned with swelling of the

erythrocytes. That the two phenomena are interrelated may be indicated by our observation that venom has no influence in expanding the erythrocytes in the absence of serum. It is well known, since the classic experiments of Flexner and Noguchi (1902), that the active principles of different venoms require an intermediate substance to make manifest their hemolytic activity. These investigators discovered that the venom of rattlesnakes and cobras had no hemolytic action on thoroughly washed erythrocytes, but that the addition of a trace of blood serum restored this function. They demonstrated that the intermediate substance had the familiar properties of serum complement. The fact that this relationship holds for the expansile influence of venom on erythrocytes, as well as for its hemolytic action, indicates that cell expansion is a precursor of hemolysis.

It is possible (although there is much evidence against this view) that the swelling of the erythrocytes under the action of venom is only a special case of the similar action of crotalin on all the tissues of the organism. As evidence for this, it may be mentioned that crotalin causes intense edema of perfused organs, and the post-mortem picture following death by crotalin is marked edema of the viscera as one of the outstanding features. It has been customary to assume that the cytolytic, the neurotoxic, the hemorrhagic, and other properties of crotalin are dependent on separate constituents of the venom. We are not convinced, from the evidence presented, that this is necessarily the case. There is remarkable uniformity in the action of crotalin on all the tissues of an animal, and it is not necessary to postulate that each tissue necessarily is attacked by a different poison.

Mitchell and Reichert (1886) found that when a drop of blood from man or other mammals was treated with venom and observed microscopically, the erythrocytes lost their biconcavity, becoming transformed into spheroidal globules. These exhibited great adhesiveness, arranging themselves in aggregations of various sizes and shapes. The corpuscles comprising these groups appeared to fuse so that their outlines became indistinct. The blood of birds, on being treated similarly with venom in vitro, manifested these changes to a much smaller degree. The nuclei underwent rapid necrotic changes, which finally resulted in granular particles, which were seen floating between the corpuscles. A number of experiments were made to study the changes in the corpuscles of living animals that had been injected with venom subcutaneously. In the case of a young cat, the blood removed near the region of injection, two minutes after injection, manifested beginning transformation of the erythrocytes into spherical globules. Thirty minutes after the injection, blood removed from the heart showed all the erythrocytes to be spherical.

We have found that the blood of the rabbit is not as amenable to the influences of crotalin as is the blood of the dog. Noguchi (1909) stated that

the blood corpuscles of different mammals present remarkable differences in their power of resisting the destructive effects of venom; those of the dog are more sensitive than those of any other animal. In the case of cobra venom, Stephens and Myers (1898) found that hemolysis occurred in a concentration of approximately 0.05 mgm. for each cubic centimeter of dog's blood. In corpuscles of the rabbit, a 2 per cent solution of cobra venom caused complete hemolysis in thirty minutes, whereas rattlesnake venom required two hours to bring about the same result.

Flexner and Noguchi (1902) made the significant discovery that venom did not have any hemolytic influence on corpuscles that had been thoroughly washed in sodium chloride solution to remove every trace of serum. However, when the separated serum was restored to each of the several kinds of blood corpuscles and treated with venom, lysis resulted. This intermediate substance had all the properties of serum complement, and the mechanism of hemolysis by venom was thus brought into relationship with that produced by serum.

Calmette, however, found that this complement differed from ordinary serum complement in that, not only did it retain its activating property by heating at 62°C., but that heating to 100°C. actually resulted in a much more powerful serum with regard to its ability to activate venom hemolysis (Noguchi, 1909).

Kyes (1902, 1903), under Erhlich's direction, found that this thermostable intermediary substance could be extracted with alcohol, and on this basis he managed to discover that of all alcohol-extractible substances present in blood, lecithin alone possessed the property of activating cobra venom in the same manner as heated serum. He finally prepared a lecithin compound of cobra venom, the hemolytic action of which was almost instantaneous, and similarly he prepared a lecithid of the venom of *Crotalus adamanteus*.

Ponder (1922), in a report on the alterations in the diameter of erythrocytes during hemolysis induced by hypotonic sodium chloride solution, showed that the erythrocytes of human beings are increased in volume from 88.5 to 131μ^3. Certain agents are capable of destroying the capsule or envelope at once, thus causing hemolysis without an increase in cell volume. Other agents have less action on the envelope and the cell expands greatly before breaking down. The effect of crotalin on the erythrocytes of the dog is analogous to Ponder's observations concerning the effect of saponin on the erythrocytes of human beings.

SUMMARY

If a sublethal dose of crotalin is injected intravenously into a dog, and samples of blood removed before and after the injection are centrifuged separately, a remarkable increase in the ratio of corpuscles to plasma is

found in the specimens taken immediately after the injection of venom, and in those taken for at least one and a half hours thereafter. This change is not due to the loss of fluid from the circulation, since immediately following the injection of venom there is no alteration in number of erythrocytes, leukocytes or platelets, and no change in the concentration of the hemolgobin. Whereas the ratio before the injection of the venom is approximately 6.2 to 8.8, the ratio immediately afterward is 10 of corpuscles to 5 of plasma.

The same change in the ratio of corpuscles to plasma is demonstrable when equivalent quantities of venom are added to heparinized blood in a test-tube. When a suspension of erythrocytes in heparinized plasma is viewed microscopically, it can be readily seen that, following the addition of a trace of crotalin to the mixture, the erythrocytes rapidly expand and become spherical. The addition of crotalin to oxalated blood in vitro is not followed by such changes. The blood must be heparinized, defibrinated, or received into oiled receptacles.

Following the increase in the ratio of corpuscles to plasma from the addition of venom in vitro, hemolysis takes place, the intensity of which is to a certain extent proportional to the increase in the ratio.

If the erythrocytes are washed thoroughly to remove all traces of plasma, the addition of venom to a suspension of corpuscles in sodium chloride solution is followed neither by hemolysis nor by an increase in the ratio of corpuscles to supernatant fluid. These occur as usual when a small amount of plasma is added to the mixture.

On the basis of these data, we have concluded that hemolysis from rattlesnake venom is due to the expansion of the erythrocytes, with accompanying injury to their membrane. The effect of crotalin on the erythrocytes of the dog is analogous to the reported effect of saponin on erythrocytes of human beings.

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THE PHYSIOLOGIC ACTION OF RATTLESNAKE VENOM (CROTALIN)

IV. The Effect on Lower Forms of Life

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In previous papers we have described the physiologic action of crotalin on the dog, rabbit and guinea pig. The effects produced on these animals were marked and they differed sufficiently to excite our interest in the possible reactions of lower forms of life to this poison. Consequently we carried out a series of experiments on the catfish, *Amieurus nebulosus*, on the frogs, *Rana palustrus* and *Rana pipiens*, and on certain protozoa. We wished to compare the action of crotalin on these animals with its effects on higher forms in the hope that some light might be shed on the fundamental nature of the action of this venom. The following experiments were done with this end in view.

When the catfish was injected with a dose of 0.3 cc. of 2 per cent venom intra-abdominally and intramuscularly it did not experience any inconvenience as judged by its behavior. After twenty-four hours, the only visible evidence of the injections was a large swelling around the site of the intramuscular puncture from the center of which a bloody exudate escaped. Around the site of the control injection, where 1 cc. of Ringer's solution had been used, swelling was not visible. The fish was alive two months later, and the lesions produced by the crotalin were still noticeable. Sloughing did not occur as is usual when the solution is injected into mammals subcutaneously but when the integument was removed in the region of the swelling the muscles were found to be destroyed and the space was full of necrotic fluid.

It was pointed out by Mitchell (1861) that frogs are highly resistant to crotalin. In general it may be stated that the frogs, *Rana palustrus* and *Rana pipiens*, were not greatly inconvenienced by doses of venom sufficient to kill a dog. Following an injection of 0.5 cc. of 2 per cent venom nothing of note occurred until about ten or fifteen minutes had elapsed, when the animal squatted with its throat resting on the table. About twenty minutes after the injection, the belly, throat and ventral surfaces of the legs became distinctly reddened. About forty-five minutes

later the redness had disappeared and the frog was again apparently normal. Larger doses of venom produced a more decided response. The following observations on *Rana pipiens* will indicate the typical reactions to doses of 0.5 cc. of 4 per cent venom.

At 4:00 p.m. 0.5 cc. of 4 per cent venom was injected into the dorsal lymph sac of a frog. Thirty seconds later the frog became inactive. There was no sign of breathing. The eyes were closed and the body limp. It was placed at once under a dissecting microscope, ventral side up. The whole ventral surface appeared normally white.

At 4:01 p.m. congested areas appeared on the throat, belly and median ventral surface of the thigh. In these areas the vessels were distended with blood. There was no evidence of hemorrhage. An examination of the web of the foot showed the veins full and circulation greatly retarded.

At 4:02 p.m. the frog became active and jumped off the stage of the microscope. Most of the injected areas were normal in color. The following morning the frog behaved normally.

A similar series of observations were made on a frog, *Rana palustrus*, into which 5 mgm. of *Crotalus atrox* venom was injected. This frog, however, died overnight.

Since these observations indicated that crotalin in sufficiently large doses produced a decided reaction in the frog we observed the effect of such injections on the capillaries. The foot of an immobilized frog was placed under a compound microscope and the following observations were made:

At 2:32 p.m. the capillaries were measured by means of an ocular micrometer; most of the capillaries were about 10μ in diameter.

At 2:36 p.m. 0.5 cc. of 2 per cent venom was injected into the dorsal lymph sac. The diameter of the capillaries did not change.

At 2:37 p.m. the circulation showed great speeding up in all vessels.

At 2:41 p.m. the speed of circulation was decreasing and the venules were distended. Blood stagnated on the venous side. Speed of circulation was subnormal in all vessels in the web of the foot.

At 2:43 p.m. contraction of some of the capillaries was apparent. Red corpuscles were held in the capillaries.

At 2:50 p.m. capillaries were from 5 to 7μ in diameter.

At 3:14 p.m. some of the capillaries were narrower near the juncture with the arterioles and venules.

At 3:28 p.m. the capillaries did not show further change.

All of our previous experiments pointed to the possibility that crotalin is nonspecific in its action on cells, that it is a protoplasmic poison. We were, therefore, interested in its effect on one-celled organisms. We investigated the influence of dilute solutions of crotalin on various protozoa, and found that certain ciliates and flagellates were killed almost instantly when placed in a dilution of 1:10,000. Others were more resistant and

succumbed after varying lengths of time. The following experiments with *Paramecium caudatum* and the amoeba *Chaos diffluvieus* will indicate the effect on two types of protozoans:

At 11:37 a.m. a drop of 1:1000 dilution of *Crotalus atrox* venom was added to a drop of water containing *Paramecium caudatum* making a dilution of about 1:2000. The organisms immediately sought the periphery of the drop.

At 11:42 a.m. the protozoans were all shrunken and were visibly collapsed, appearing flat rather than cylindrical.

At 11:50 a.m. most of the Paramecia continued to move actively about, many of the movements being in a vertical direction, and lacking coördination. A few spun around without progressing, and a few had become immobile.

At 12:00 m. nearly all of the Paramecia had become distended. They appeared much as they would if placed in distilled water. Many were clumped together, there being as many as eight in a clump.

At 12:10 p.m. all the Paramecia were swollen. Many had disintegrated.

It required thirty-five minutes for a dilution of 1:10,000 of crotalin to destroy *Chaos diffluvieus.* At the end of that time the amoeba had become altogether inactive and the protoplasm had taken on a congealed appearance.

Philpott (1929), reported the lethal action of *Crotalus atrox* venom on *Paramecium caudatum* and other protozoa.

COMMENT AND CONCLUSIONS

Flexner and Noguchi (1901–1905) made a careful study of snake venoms in relation to bacteriolysis. On the basis of their observation that venom destroyed bacteria, they concluded that venoms contained a cytolysin. It has also been stated that crotalin contains a hemolysin, a hemorrhagin, a neurotoxin and other substances. Our observations on the action of crotalin suggest that crotalin is a poison to protoplasm that kills protoplasm wherever it comes in contact with it. The action of crotalin on the Paramecium is strikingly like its action on erythrocytes. In previous studies of this series we reported its destructive action on the rabbit's heart, on the gastrocnemius muscle of the frog and on the uterus of the guinea pig. In each instance the results were such as to lend support to the conception that crotalin is a nonspecific protoplasmic poison.

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THE PHYSIOLOGIC ACTION OF RATTLESNAKE VENOM (CROTALIN)

V. Some Experiments on Immunity to Crotalin

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In previous papers we have pointed out the marked depressor action of rattlesnake venom (crotalin) in dogs and rabbits and also its characteristic effect on the erythrocytes of the dog when injected in vivo or added to the blood in vitro. These results are invariably so pronounced that we have considered them as highly satisfactory criteria in the measurement of the degree of immunity developed in dogs following repeated injections of crotalin.

Experimental observations. A number of preliminary experiments indicated that dogs could be given from 0.1 to 0.2 cc. of 2 per cent crotalin intravenously without serious injury. A series of dogs was injected with doses ranging from 0.1 to 0.6 cc. of 2 per cent crotalin. Following each injection until about the sixth or seventh, the animals manifested more or less severe reactions, as indicated by vomiting and defecation. The symptoms, however, became progressively milder, so that the dosage could be safely increased after six or seven injections. At the end of five or six weeks the dogs did not show visible reaction to as much as 0.5 cc. of 2 per cent venom injected intravenously. Such a dose is usually fatal to a normal dog. The pulse rate proved to be a reliable criterion of the effect of the venom. When this remained relatively constant before and after the injection of a given dose of crotalin the size of the dose was increased (table 1).

March 3, a record of the blood pressure of the first dog was made. The animal, when injected previously with 0.5 cc. of 2 per cent venom, had not been inconvenienced. Following three intravenous injections totaling 1 cc. of 2 per cent venom the blood pressure did not fall (fig. 1). March 15 the blood pressure of the second dog was recorded. At this time 2.4 cc. of 2 per cent venom was injected in six successive doses of 0.4 cc. each. After each dose there was a slight fall which was followed by an immediate return to the normal level. A record of the blood pressure

of the third dog of the series was taken March 7. An injection of 0.3 cc. of 2 per cent venom exerted a temporary depressor action which was almost immediately followed by a return to normal (fig. 2). It will be clear that a definite conception of the degree of immunity to a given amount of crotalin may be obtained by studies on the blood pressure of animals.

During these investigations on the blood pressure we studied the effect of crotalin on the blood. At the beginning of the experiment 15 cc. of blood was drawn from each animal into each of two tubes. Sufficient heparin was placed in the tubes to prevent clotting for four hours. To

TABLE 1

FIRST DOG, 25 KGM.		SECOND DOG, 20 KGM.		THIRD DOG, 13 KGM.	
Date	Cc. of 2 per cent crotalin injected daily	Date	Cc. of 2 per cent crotalin injected daily	Date	Cc. of 2 per cent crotalin injected daily
January 22–23	0.1	January 22–25	0.1	February 6	0.21
January 24–25	0.2	January 26–30	0.1	February 8–9	0.2
January 26–31	0.3	January 31	0.3	February 11–12	0.25
February 1	0.15	February 1	0.15	February 14–15	0.25
February 4	0.35	February 4–7	0.15	February 18	0.15
February 5	0.4	February 8–9	0.17	February 19–28	0.2
February 6	0.4	February 11	0.2	March 2	0.2
February 7–9	0.45	February 12	0.25	March 4–8	0.3
February 11–12	0.5	February 14	0.25	March 11–13	0.4
February 14	0.6	February 15–16	0.15	March 14	0.5
February 15–18	0.3	February 18	0.2	March 15	0.4
February 19–21	0.4	February 19–23	0.3	March 18	0.5
February 22–26	0.45	February 25	0.3		
March 6	0.5	February 26	0.3		
		February 27	0.35		
		February 28	0.4		
		March 2	0.4		
		March 4–8	0.4		
		March 11	0.5		

one of these an amount of venom proportional to the size of the dose injected was added. After the tubes were centrifuged for fifteen minutes the ratio of corpuscles to plasma was greatly increased in the tube to which the venom was added. As immunity developed the ratio of the corpuscle to plasma gradually decreased. When the dog had become immune to a given dose of venom there was no change in the ratios of the two tubes. March 3 the first dog showed total immunity to a dose of 1 cc. of 2 per cent venom (fig. 1). Two tubes of blood were taken before the injection and to one of these 0.01 cc. of 2 per cent venom was added. After centri-

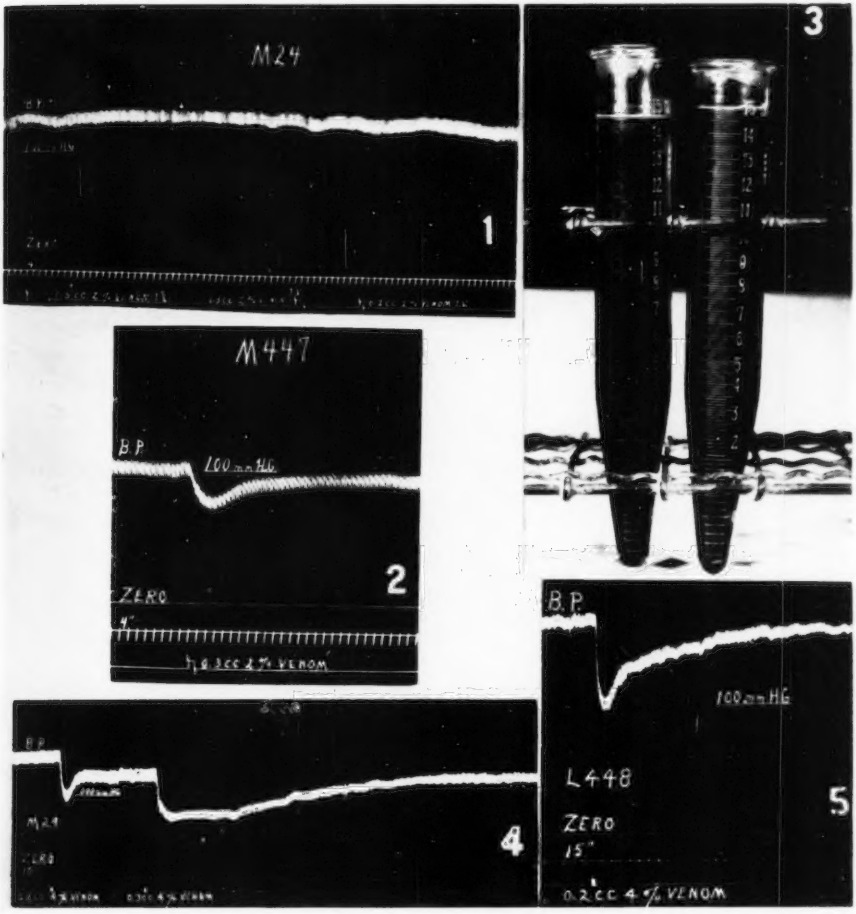

Fig. 1. Blood pressure of the first dog of the series which had become immune to a total of 1 cc. of 2 per cent crotalin.

Fig. 2. Blood pressure of the third dog of the series. The animal was almost immune to 0.3 cc. of 2 per cent venom.

Fig. 3. The tube shown on the left contains normal blood from the first dog of the series. To the tube on the right 0.01 cc. of 2 per cent crotalin was added. Both tubes were centrifuged fifteen minutes.

Fig. 4. Blood pressure fifty-five days after injections of crotalin were discontinued (first dog). At this time 0.5 cc. of 4 per cent crotalin had a marked depressor effect.

Fig. 5. Blood pressure of the second dog thirty-five days after injections of venom were discontinued. An injection of 0.2 cc. of 4 per cent crotalin produced a marked reaction.

fuging fifteen minutes the blood corpuscles in both tubes stood at the same level (fig. 3). We obtained the same results when one sample of blood was taken from the dog before and one immediately after the injection of crotalin.

Since we were interested in ascertaining the duration of the immunity acquired by these animals all injections were discontinued. The first dog did not receive crotalin after March 7 until fifty-five days had elapsed; then a record of the blood pressure was made and the ratio of corpuscles to plasma was determined. An injection of 0.2 cc. of 4 per cent crotalin caused a decided fall in blood pressure, with partial recovery. A second injection of 0.3 cc. of 4 per cent venom produced a further fall with a much slower recovery (fig. 4). The ratio of corpuscles to plasma in the control tube was 7.5 to 7.5 cc., while the tube to which 0.005 cc. of 2 per cent crotalin was added showed a ratio of 12.5 to 3.5 cc. The second dog was allowed to go thirty-five days after the last injection, March 7. A marked fall resulted from an injection of 0.2 cc. of 4 per cent venom. Rapid recovery followed (fig. 5). The change in ratio of the corpuscles to plasma was the same as that described for the first dog's blood. The third dog did not receive further injections after March 29 until twenty-five days had elapsed, then a record of the blood pressure was made. There was only a temporary depressor action following an injection of 0.2 cc. of 4 per cent venom. The ratio of corpuscles to plasma was 5.3 to 9.7 cc. for the control and 9.7 to 5.3 cc. for the tube to which 0.005 cc. of 2 per cent crotalin was added.

SUMMARY AND CONCLUSIONS

These experiments indicate that blood pressure can be used as a satisfactory criterion of the degree of immunity acquired by dogs after the injection of a series of graduated doses of venom. If the blood pressure is uninfluenced by a strongly depressor dose of crotalin the animal may be considered to be immune. This conclusion is borne out by the observation that dogs do not suffer untoward effects from doses of crotalin sufficient to kill a normal animal. The degree of immunity acquired is apparently greater in those animals that have received more venom. The ratio of corpuscles to plasma may likewise be used successfully as a measure of acquired immunity in dogs. As judged by these criteria, the immunity to crotalin acquired by dogs is of comparatively short duration.

THE ANAEROBIC OXYGEN DEBT OF FROG NERVE

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It is well known that living tissues when brought into anaerobic conditions are able to carry on their vital processes for some time by means of the energy derived from fermentation. As a result of these fermentation reactions there develops an oxygen debt. One possible measure of the oxygen debt is the extra amount of oxygen, over and above the basal requirement which the tissue will consume when again brought into aerobic conditions. Few quantitative data are however available concerning the magnitude of this oxygen debt in different tissues and under different conditions. In this paper I propose to summarize some experiments which have been undertaken in the last two years with the object of measuring this oxygen debt in frog nerve. In a later paper the results of similar experiments with frog muscles will be communicated.

METHOD. The nerves are placed in a respirometer of the differential volumeter type. They are in contact at the central end with a set of platinum electrodes for stimulation and a set of silver electrodes at the peripheral end for recording the negative variation in response to a tetanus. A slow moving (20 sec.) D'Arsonval galvanometer of high current sensitivity is used for recording and the deflection serves as a rough index of the functional condition of the nerve. The oxygen consumption is first measured in a preliminary period after which nitrogen or hydrogen, purified by passing over hot copper and moistened by passing through water, is conducted through the nerve chamber by all copper and glass tubes with connections of DeKhotinsky cement. After a period in nitrogen, air is admitted to the nerve chamber and measurements of the oxygen consumption are resumed. This constitutes the difficult part of the technique for the slightest variation in vapor pressure or temperature of the new gas mixture will cause movements of the index drop which is supposed to respond only to changes in oxygen. The maximum error due to the introduction of air may fairly be put at 0.5 volume per cent. The gas to be introduced is previously stored over mercury and isotonic sodium hydroxide in the same water bath with the respirometer. The cocks to the respirometer are opened and the new gas mixture is made to pass through the nerve chamber by displacement with mercury for 10 seconds. The

respirometer cocks are then immediately turned so that volume readings can be made. Details of this technique have been described previously in connection with measurements of CO_2 absorption (1). Since oxygen is more soluble in water than is nitrogen, there is always a shrinkage of volume when nitrogen is replaced by oxygen even if no nerve is present. To minimize this change, CO_2-free air is used instead of pure oxygen and a very small amount of sodium hydroxide (0.1 to 0.2 cc.) is used to absorb carbon dioxide. When hydrogen is used in place of nitrogen there is always a considerable increase of volume when air is first introduced due to the escape of hydrogen being more rapid than the solution of air in the nerve and the sodium hydroxide. Fortunately at the experimental temperature (22°C.) the solubility of hydrogen in water (0.0179) is practically identical with the solubility of air (0.0180) so that the final change of volume from this cause is nil. In some experiments measurements have been made simultaneously of the carbon dioxide evolution from the nerve by absorbing the CO_2 in barium hydroxide, the electrical conductivity of which was then determined (2). This procedure gives valuable information concerning the carbon dioxide but makes the measurement of the oxygen debt more difficult on account of the large volume of barium which must be used (at least 1 cc.) thus making the correction for solution of gases rather large.

Results. The existence of an anaerobic oxygen debt in nerve was demonstrated by Gerard (3), who stated that it was 1.5 volumes per cent greater after two hours in nitrogen than after half an hour. His method of introducing air was not such that the total oxygen debt could be accurately measured. After a preliminary series of experiments I published details of one experiment (4) showing a considerably larger debt of 10.1 volumes per cent (see experiment 21, table 1). Subsequent experiments have indicated that this result was exceptional though not impossibly large as table 1 will show.

In table 1, the experiments are grouped in four series according to the time of year and there seems to be some seasonal correlation discernible, the average oxygen debt (last column) in June being 1.5 volumes per cent increasing to 2.0 volumes per cent in August and to 4.3 in the autumn. This last value is nearly as high as the figure 4.7 volumes per cent obtained the preceding January but not as high as the figure of 9.4 and 10.1 volumes per cent obtained in what seemed to be, at the time, the most reliable of these early experiments. It may be noted however that the variations in this January series are so great that taking an average is hardly justifiable. This variation is largely connected with the fact that these experiments involved the simultaneous measurement of both CO_2 and O_2, which necessitated 1 cc. of $Ba(OH)_2$ inside the nerve chamber and hence a large correction for volume change due to solution of oxygen, as already ex-

plained. There may be other undefined reasons for the variation due to my early inexperience with the technique. The frogs used for the experiments of table 1 were almost all kept in the cold room (5–10°C). for some time (a few days to a month) before being used, even in the case of summer frogs. I was not able to establish any difference by this method between nerves from such frogs and nerves from frogs kept at room temperature or freshly brought in from out of doors, as some of them were. The only correlation seemed to be with the season of the year and they have been grouped accordingly. In some experiments I tried to increase the oxygen debt by soaking the nerves in alkaline Ringer's solution buffered with phosphates, but no effect could be established.

A few other points concerning table 1 require comment. CO_2 was not simultaneously measured except in the experiments of January 1928. Hence the correction for dissolved oxygen replacing nitrogen was only about 0.5 volume per cent. In most cases, however, the nerves were in contact with stimulating and leading off electrodes (Ag-AgCl) so that the time necessary for complete disappearance of the electric response could be measured. This figure (asphyxia time), where available, is noted in the third column, table 1. It is seen to increase more or less in proportion to the oxygen debt, the average time being 1.7 hours in June, and increasing to 3.1 and 3.6 hours in August and October respectively. Such a relationship might reasonably be expected. In experiments 1 and 2 in June the nerve could be completely asphyxiated and revived 3 and 4 times respectively. It will be seen that the time needed for asphyxia decreases from 1.4 hours during the first nitrogen period (experiment 2) to 0.4 hour during the fourth.

In the fourth column of table 1 are given the volumes of oxygen which the nerve would have consumed during the anaerobic period at the previous (or sometimes the subsequent) basal rate. Only a relatively small fraction of this "O_2 missed" is made up for afterwards. This fraction is larger when the anaerobic period is shorter indicating that most of the oxygen debt accumulates during the early part of the anaerobic period. No correlation can in fact be made out from table 1 between the duration of the anaerobic period and the magnitude of the oxygen debt. Since the average durations of the anaerobic periods in the 4 series of experiments did not differ appreciably it is to be expected that the per cent of the O_2 missed during nitrogen which is made up during recovery should be low (7.7 and 8.7 per cent respectively) in June and August when the oxygen debt is low and should increase during autumn and winter to 14.7 and 22.8 per cent as the oxygen debt increases. Such figures seem to indicate that the nerve markedly diminishes its energy turnover in nitrogen. It should perhaps be remembered, however, that the "oxygen debt" as measured is, to be exact, only that portion of a true oxygen debt which is "paid off"

TABLE 1

Oxygen debt of nerve after anaerobiosis

EXPERIMENT	ANAEROBIC PERIOD	ASPHYXIA TIME	O_2 MISSED	O_2 DEBT
		June, 1928		
	hours	*hours*	*vols. per cent*	*vols. per cent*
1	3.0	1.3	20.8	1.0
	1.3	0.5	15.1	2.3
	1.5	0.4	17.4	1.3
2	1.4	1.4	7.7	1.6
	1.0	1.1	5.5	1.5
	1.0	0.9	5.0	1.5
		0.4		
3	6.3	3.0	29.6	2.7
4	4.5	3.0	21.8	1.6
5	8.5	3.0	38.6	0.4
6	3.5		10.4	1.8
	2.3		6.7	1.3
	10.0		29.2	2.2
7	2.6	2.2	14.4	1.0
	10.0		49.5	1.4
Average		1.7	19.4	1.5
		O_2 debt = 7.7 per cent of O_2 missed		
		August, 1928		
8	3.5	3.2	28.4	1.5
9	9.0	6.0	22.3	2.4
10	2.5	1.7	22.6	3.0
11	4.5	1.7	18.6	1.2
Average		3.1	23.0	2.0
		O_2 debt = 8.7 per cent of O_2 missed		
		October–December, 1928		
12	8.5		41.0	2.9
13	15.0		49.0	4.6
14	4.5		20.8	5.9
15	4.2	3.7	15.2	4.8
16	6.4	3.5	24.1	3.9
17	8.0		25.0	4.0
Average		3.6	29.2	4.3
		O_2 debt = 14.7 per cent of O_2 missed		

TABLE 1—*Concluded*

EXPERIMENT	ANAEROBIC PERIOD	ASPHYXIA TIME	O_2 MISSED	O_2 DEBT
Preliminary experiments, January, 1928				
	hours	*hours*	*vols. per cent*	*vols. per cent*
18	7.0	4.5	27.3	2.9
19	2.7	4.0	8.4	3.7
20	4.7	3.7	19.9	0.4
21	2.4	1.3	18.7	10.1
22	8.6	3.0	41.0	9.4
23	4.0	4.0	8.4	1.5
24		4.8		
25		4.3		
Average		3.6	20.6	4.7
O_2 debt = 22.8 per cent of O_2 missed				

during recovery. The fact that lactic acid accumulates in nitrogen without disappearing in recovery (Gerard and Meyerhof, 1927) indicates that the true oxygen debt may be larger than the figures of table 1 indicate.

Usually the oxygen consumption returns to what is clearly a basal level after recovery, and this is used as a base line in calculating the oxygen debt. The question arises, however, whether a control nerve, kept in oxygen all day without administration of nitrogen would not have consumed oxygen at a still lower rate, in which case the oxygen debt has been underestimated. This question is easily answered in the negative by a control experiment like that plotted in figure 1. The two lower graphs represent the rates of O_2 consumption of the control nerve (dotted) kept continuously in air and of the anaerobic nerve which is in hydrogen for 4 hours (solid line). The experiment shows that whereas the control nerve started with a slightly lower rate of oxygen consumption than the anaerobic nerve it ended up with a higher rate. Nitrogen treatment apparently diminished the ability of the nerve to utilize oxygen or its need for oxygen. A repetition of this experiment gave exactly the same result. There is therefore no reason to distrust the base line used in calculating the oxygen debt.

Some of Gerard's experiments (3) led him to suggest that after nitrogen there was some delay before the oxygen consumption regained its normal value. The reverse seems to be the case in my experiments, the maximum value being attained during the first few minutes.

The upper graph in figure 1 represents the negative variation of the nerve in response to tetanic stimulation (continued to maximum deflection or for 10 seconds) as recorded on a slow moving D'Arsonval galvanometer. This graph shows the marked increase in the electrical response

as the asphyxia progresses which is characteristic of practically every such experiment. (It may possibly be absent in acid-soaked nerves.) After

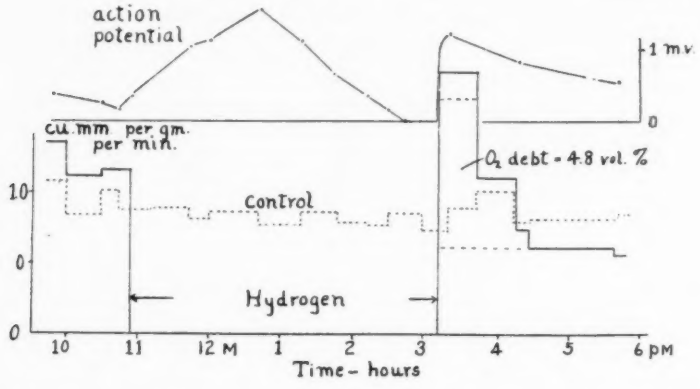

Fig. 1. The oxygen consumption rates of two sets of nerves, one kept in air all day (control, dotted line) and the other put into nitrogen for a period of 4.3 hours (solid line). The latter shows an excess oxygen consumption after air is readmitted of 5.3 volumes per cent of which 0.5 is attributed to solubility of air in the nerve and the sodium hydroxide in the apparatus. The upper graph shows the negative variation of the nerve which was asphyxiated.

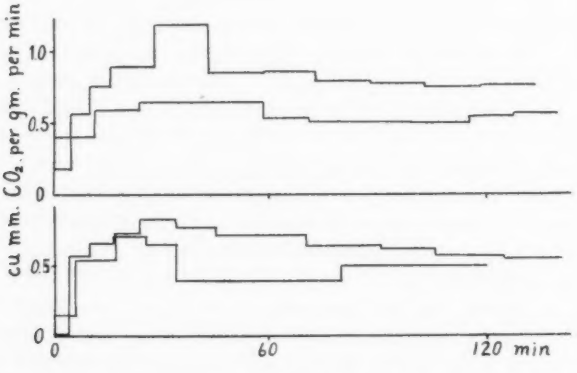

Fig. 2. Graphs from 4 experiments showing that the rate of carbon dioxide output immediately after the admission of air to an asphyxiated nerve may pass through a maximum. Measurements were made by the conductivity of barium hydroxide method.

recovery the deflection passes again through a maximum. This maximum suggests an optimum rH or cH which the nerve attains at some point in-

termediate in both asphyxia and recovery. Davis, Pascual and Rice (1927) have shown a similar maximum in the electrical response of nerve when its acidity is progressively increased by CO_2 administration. In this connection an interesting feature of one of these experiments deserves special mention. A nerve had been asphyxiated once and had recovered in the usual way. It was then asphyxiated again with nitrogen and left in nitrogen over night. In the morning air was administered and an oxygen debt of 1.9 volumes per cent was recorded. At the same time the negative variation reappeared to about $\frac{1}{2}$ to $\frac{1}{3}$ its previous value and then disappeared again completely one hour after the air had been admitted. Unfortunately no attempt was made to see whether the electrical response could be revived again by further administration of N_2 which might have brought the cH or rH again to an optimum value.

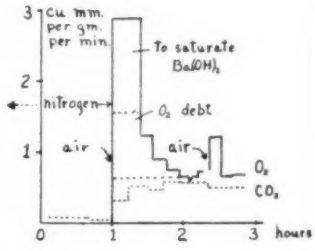

Fig. 3. Oxygen consumption (solid line) and CO_2 output of nerves when air is admitted to the nerve chamber after 7 hours in nitrogen. A large correction of 3.4 volumes per cent is made for the diminution of volume caused by the saturation of $Ba(OH)_2$ with air instead of nitrogen.

I have already reported (4) one rather important feature of these experiments, i.e., the fact that the rate of CO_2 output during recovery from nitrogen may occasionally be observed to pass through a maximum, and I have pointed out above the significance of this fact. As further confirmation might seem necessary, however, attention may be called to the four experiments recorded in figure 2 in which the rate of CO_2 output during recovery from nitrogen is plotted against time as measured from the moment when nitrogen was replaced by air. In each case the rate of CO_2 output passes through a maximum at the end of 20 to 40 minutes. Granted this fact there seems no escape from the conclusion that *part at least of the processes underlying the oxygen debt of nerve must involve the formation of* CO_2. A similar maximum, observed in the case of muscle, is explained by the burning of lactic acid. Of course there might be a formation of CO_2 during recovery which was so masked by CO_2 retention that no maximum would be observed. The absence of a maximum therefore in a number of other similar experiments does not throw doubt upon this conclusion.

The CO_2 content of nerve before and after recovery from nitrogen. The essential feature of the recovery gas exchange after nitrogen is that the oxygen is very much greater than the carbon dioxide; the R.Q. is low. Its actual value of course depends upon the time interval chosen for measurement. This was reported in my preliminary experiments and has been

confirmed by Schmitt (1929) by a different method. The graph of figure 3 taken from experiment 18 of table 1 illustrates this relationship. The nerves were kept in pure nitrogen for 7 hours when air was introduced. The subsequent rate of CO_2 production as shown by the dotted line is much lower than the oxygen consumption. As a control measure air was similarly introduced a second time after 1.4 hours of recovery, as indicated by the arrow, with comparatively little disturbance in the rate of oxygen consumption.

In comparison to the oxygen there is therefore some expected carbon dioxide which does not appear. This must be either retained in the nerve or must be non-existent. The fact that Schmitt found a low R.Q. by his method which included the measurement of the carbon dioxide within the nerve by acidification suggests the latter interpretation. I have en-

TABLE 2

Combined CO_2 contents of nerves in volumes per cent before and after recovery from anaerobiosis

EXPERIMENT	BEFORE RECOVERY	AFTER RECOVERY	CORRECTED AFTER RECOVERY
1	15.5	14.1	14.4
2	12.9	10.2	10.6
3	10.3	7.5	7.8
4	16.2	15.9	16.5
5	11.7	13.2	13.6
6	9.7	9.1	9.8
7	17.5	15.5	16.6
8	11.1	9.2	10.1
Average.....	13.1	11.8	12.4

deavored to confirm this conclusion (i.e., that the missing carbon dioxide is not retained within the nerve) by measurements of the carbon dioxide content of the nerve before and after recovery from a period in nitrogen. I have done this by two methods.

a. First method: Two sets of nerves were equilibrated with 5 per cent carbon dioxide in nitrogen and left for four hours at the end of which time acid was dumped on one set of nerves and the carbon dioxide evolved was measured while 5 per cent carbon dioxide in oxygen was admitted to the other nerve. At the end of two or three hours' recovery, acid was dumped on the second set of nerves, and the carbon dioxide evolution likewise determined. The results are collected in table 2 and show that under these conditions there is, if anything, less carbon dioxide after recovery than before.

Before the anaerobic nerve is acidified the index drop is moving away from the nerve bottle, indicating an evolution of anaerobic CO_2. After acidi-

fication this movement is increased for a time and then ceases and the drop remains stationary for hours. In a nerve recovering in oxygen, however, the drop is moving toward the nerve bottle before acidification, due to retention of CO_2 in the nerve and to an R.Q. less than 1.0. After acidification the drop of course moves away from the nerve bottle for about half an hour but then resumes for a time its former movement toward the nerve bottle. Presumably the sulphuric acid used for acidification (0.2M) does not stop the oxygen consumption completely. Correction for this movement tends to increase the combined CO_2 in the recovery nerve as shown in table 2. Even with this correction, however, there is no evidence of increased combined carbon dioxide after recovery.

For each of these experiments I have used nerves of 2 or 3 frogs. The nerves were divided into 2 groups, each frog contributing one nerve to each group. Each group was carefully weighed on a torsion balance and put on a shelf in a small respirometer bottle of Pyrex glass of about 7cc. capacity Five-tenths cubic centimeter of a solution of 0.2M H_2SO_4 (sometimes HCl) was put in the bottom of the bottle. Thus the nerve could be acidified by tipping the apparatus until the acid covered the shelf. Only when this technique was adopted could consistent results be obtained. The nitrogen used for these experiments was purified over hot copper with other precautions as described above. Care was taken also to pass nitrogen through the capillary tube of the respirometer to remove all oxygen. In doing this the kerosene index drop was of course broken but with a little experience there is no difficulty in reassembling it.

Twelve other experiments were carried out previous to those reported in table 2 with slightly different technique. In these experiments acid was placed in a central well in the bottle, (which was of soft glass), the nerve being in the annular space outside. In 5 of these experiments the nerve was in Ringer's solution. Control experiments with this apparatus showed that the acid could not be dumped out of the well into an otherwise empty bottle (no nerves) without causing serious disturbances of the index drop. This was obviously the reason for the considerable irregularity of the results obtained which in consequence had to be discarded. Only by the changes described above could consistent results be obtained. Even so these early results were not very different from those of table 2 the average CO_2 contents being 17.1 volumes per cent before recovery and 18.3 volumes per cent after.

b. Second method: By the second method an attempt was made to determine whether the carbon dioxide content of the nerves *at zero CO_2 tension* would increase during recovery. This attempt was prompted by the possibility that a high CO_2 tension might inhibit the recovery and make results obtained by the first method fallacious. To maintain zero CO_2 tension the carbon dioxide evolved is absorbed in sodium hydroxide contained in the bottle until the moment when acid is to be dumped; then the sodium hydroxide is removed from the bottle by a turn of a stop cock. Any further evolution of carbon dioxide must accumulate in the bottle and cause the index drop to move. This method which need not be described in detail at this time consists essentially in introducing sodium hydroxide

into the nerve bottle through a side tube containing close to the bottle a stop cock of large bore. The sodium hydroxide meniscus comes just to the middle of this stop cock and is secured by another stop cock further away from the bottle. A quarter turn of the stop cock containing the meniscus removes all sodium hydroxide from the chamber.

By this method the nerves are under conditions exactly comparable to those obtaining during the experiments in which I measured simultaneously the oxygen and the carbon dioxide, the latter by the conductivity of barium hydrate. Eight experiments of this type were tried and the results are listed in table 3. If the figures are averaged there is found to be 2.05 vols. per cent of CO_2 before recovery and 2.08 vols. per cent

TABLE 3

CO_2 content of nerve after anaerobiosis

EXPERIMENT	BEFORE RECOVERY	AFTER RECOVERY
	vols. per cent	*vols. per cent*
1	2.6	3.5
2	0.9	2.8
3	3.0	1.2
4	1.3	1.2
5	.9	1.9
6	2.0	1.9
7	1.1	1.8
8	4.6	2.3
Average	2.05	2.08

after recovery, or a practical identity. Four of the eight experiments, however, showed an increase in CO_2 after recovery and four showed a decrease. This unsatisfactory lack of uniformity must be attributed to the error of the method and the very small volume changes to be measured. The actual deflections of the index drop caused by acidification of the nerve were only 3 to 10 mm., and it is difficult to "dump" acid in an empty bottle without some small volume change. All the figures of table 3 have been corrected as described for table 2. It is at least evident from table 3 that the original CO_2 content in pure oxygen of 8 to 10 volumes per cent is not regained during recovery, nor is the equivalent of the anaerobic CO_2 formation retained.[1]

[1] This anaerobic CO_2 formation was measured by the conductivity of barium hydrate method in 8 experiments. The results in volumes per cent were 12.0, 5.6, 22.0, 9.5, 10.9, 13.9, 5.1, and 8.8, the average being 11.0 volumes per cent. The method is not quite certain for systematic comparisons between the anaerobic CO_2 formation and the preformed CO_2 since errors are introduced while oxygen is being replaced by nitrogen and it takes some time to get all the oxygen out of the barium.

Comparison with frog skin. By way of comparison with frog nerve some similar measurements have been made of the oxygen debt of frog skin after anaerobiosis. The results are collected in table 4. In this respect there is no obvious difference between the behavior of frog skin and of frog nerve. When the CO_2 output was measured on frog skin simultaneously with the oxygen consumption there was the same formation of anaerobic CO_2 and the same discrepancy between the recovery carbon dioxide and the recovery oxygen. Thus in a 3 hour recovery period in air, following 3 hours in hydrogen, the CO_2 used was 19.3 volumes per cent and the oxygen 38 volumes per cent, the R.Q. being therefore 0.51. The absolute magnitudes of the oxygen debts are about the same as in nerve (1.6 to 4.6 volumes per cent) as shown in table 4. In this table the experiments have been arranged in order of the duration of the anaerobic periods. The last column shows that, as would be expected, the oxygen debt forms

TABLE 4

Oxygen debt of frog skin after anaerobiosis

(1) EXPERIMENT	(2) HOURS IN NITROGEN	(3) O_2 MISSED	(4) O_2 DEBT	RATIO $\frac{(4)}{(3)} \times 100$
		vols. per cent	*vols. per cent*	
1	0.5	7.2	2.0	27.8
2	1.0	13.2	3.4	25.7
3	2.1	15.6	1.6	10.4
4	2.8	29.3	3.2	10.9
5	4.0	53.0	4.6	8.7
6	4.1	69.0	3.5	5.1

a large part (25 to 28 per cent) of the oxygen missed when the anaerobic period is short and a small percentage (5 to 10 per cent) when the anaerobic period is long.

It will be shown in a later paper that in comparison to skin and nerve a frog muscle is very different in that the anaerobic oxygen debt may be many times larger than the oxygen which was missed.

Discussion. During a period of anaerobiosis of about 4 hours a nerve becomes completely asphyxiated and during this time there is a decrease of the combined carbon dioxide (at 5 per cent CO_2) from about 30 volumes per cent to 13.1 volumes per cent. During recovery the function of the nerve returns in spite of the fact that there is no return of the original CO_2 combining capacity. This finding is consistent with the fact that during recovery there is an excess oxygen consumption or oxygen debt which is only about 10 to 15 per cent of the oxygen which the nerve would have consumed during the anaerobic period had oxygen been available. The true oxygen debt is larger than the "paid off" oxygen debt. During recovery

the R.Q. is very low; the oxygen is in excess of the carbon dioxide by about the same amount that it is in excess of the basal rate of oxygen consumption. Hence the magnitudes of the oxygen debts may be taken as a rough measure of the amount of carbon dioxide which would be expected, on the basis of the observed oxygen consumption, but which does not appear. Since this missing carbon dioxide does not leave the nerve and apparently cannot be found in the nerve after recovery (there being no increase in the combined carbon dioxide after recovery), it must be concluded that *the missing carbon dioxide is not formed at all.* This confirms the opinion of Gerard and Meyerhof (1927) and of Schmitt.[2]

There is but one way in which this conclusion can be reconciled with the curves of figure 2 which show that the carbon dioxide output may pass through a maximum coinciding with the maximum of oxygen intake in recovery. The excess oxygen in recovery over and above the basal oxygen may be due both to the replenishment of an oxidation reserve and to the oxidation to the carbon dioxide stage of the products of anaerobic metabolism; the recovery oxygen in excess of the recovery CO_2 must be due to the former process only.

If the oxygen debt were all used for the removal of lactic acid one might expect to find an increased carbon dioxide combining power after recovery from nitrogen. The increase to be expected on this basis is however not large. Suppose the oxygen debt is 3 volumes per cent all of which is used to burn lactic acid at an oxidative quotient of 4. Under these conditions an amount of lactic acid equivalent to 4 volumes per cent of CO_2 would disappear from the nerve. If half of this lactic acid had been neutralized by bicarbonate then only 2 vols. per cent of CO_2 would be retained, a rather small difference to measure.

Failure to find an increased combined CO_2 content after recovery does not of itself indicate, however, that lactic acid is not removed, for phosphocreatine is simultaneously being resynthesized (Gerard and Wallen, 1929), which would increase the acidity. From Gerard and Wallen's data (their table 4) it appears that there is an increase of 3 mgm. P as phosphocreatine in 100 grams of nerve after recovery. This corresponds approximately to 1×10^{-4} mols of phosphocreatine which according to Fiske and Subbarrow (1929) (their table 4) at pH 7.0 would cause the removal of 5.0×10^{-4} mols of base. If all this base had been combined with carbon dioxide this would involve a decrease of 1.1 volumes per cent of carbon dioxide which is evidently a significant amount in comparison to the oxygen debts and might mask a lactic acid removal.

[2] Since this paper was sent to press Schmitt's full paper has appeared. Bioch. Zeit., 1929, ccxiii, 443.

SUMMARY

1. After a period in nitrogen a nerve consumes an extra amount of oxygen which, however, is less than $\frac{1}{4}$ and frequently less than $\frac{1}{10}$ of the oxygen of which it was deprived by the administration of nitrogen.

2. This anaerobic oxygen debt increases from values of 1.5 and 2.0 cc. per 100 grams in June and August to 4.3 and 4.7 cc. per 100 grams in autumn and winter respectively.

3. The duration of anaerobiosis necessary for the disappearance of the action current is also greater in autumn and winter (3.6 hours) than in June and August (1.7 and 3.1 hours respectively).

4. The rate of CO_2 output may pass through a maximum during recovery from anaerobiosis indicating that part of the excess oxygen used in recovery is involved in some process leading to CO_2.

5. The CO_2 content of nerve after recovery from nitrogen is if anything slightly less than before recovery, indicating that most of the oxygen debt is due to an oxidation reserve.

6. Some similar measurements of the oxygen debt of frog skin are reported which show that this tissue behaves much like frog nerve after anaerobiosis.

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BLOOD REGENERATION IN SEVERE ANEMIA

XVI. Optimum Iron Therapy and Salt Effect

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We hope that the characteristic experiments tabulated below will support two points which may be stated as follows: *First*, there is an optimum intake of iron by mouth which will produce a maximum hemoglobin regeneration in experimental anemia due to bleeding in dogs. Under the conditions of our experiments this intake amounts to about 40 mgm. of iron as metal per day. Above this optimum intake level excess iron has no appreciable effect. *Second*, there is a distinct "salt effect" by which term we mean that a variety of salts including iron, alone or combined, exert an influence upon hemoglobin regeneration without becoming incorporated in the end product—this may be designated also as a "catalytic" effect.

If some patient reviewer should attempt to read all the published literature dealing with iron effects in anemia, both experimental and clinical, he would be busied for weeks or even months. The casual reader may inquire how all this work could be done and yet the internal body metabolism of a simple metal like iron remain in dispute. But iron is an elusive sprite which mocks the investigator and will probably furnish many interesting new puzzles for students in the years to come.

There is a growing interest in iron therapy in various human anemias which are notoriously complex and demand much critical study and analysis. We may cite merely recent papers by Keefer and Yang (4), Murphy and Powers (5), who report favorable reactions in various anemias due to iron alone or iron plus liver or diet therapy.

As we have been misquoted occasionally it may be well to outline in a few words our earlier experiments with iron therapy in experimental anemia. In our first experiments (11) with *short anemia periods* we could find no evidence that iron therapy was of any value. But in subsequent experimental work (12) with *long continued severe anemia* we could bring definite evidence that iron was potent. These observations have been amply confirmed by other investigators as reviewed elsewhere (12). It is highly probable that in the short anemia experiments there was a reserve

storage of iron and other hemoglobin building stones not exhausted by the few bleedings. As this reserve was adequate for the emergency needs of the experiment it obliterated the iron effect which is so easily demonstrated in the long term anemias.

This reserve storage of hemoglobin building stones may be considerable and may require weeks of continued bleeding to exhaust it completely. It may amount to 250 to 350 grams of hemoglobin and depends in part on the diet given the growing dog. This reserve is discussed more fully under methods. It is quite important that this fact be recognized else the investigator may attribute a favorable hemoglobin output to a given diet rather than to this reserve storage. When this reserve is once exhausted the dog reacts quite uniformly to the standard ration or to various meat products and drugs as previously reported.

We have recently published experiments (7) to show that the iron effect and the liver or kidney feeding effect may often be superposed one upon the other. If the iron effect is 40 grams hemoglobin and the liver effect is 80 grams hemoglobin we may see a total output of 110 to 130 grams hemoglobin when both liver and iron are fed during the two week period. This would seem to minimize the effect of the small amount of iron contained in the liver tissue, yet much has been written about the potency of food or organic iron as contrasted with inorganic iron—for example, ferrous carbonate or ferric chloride. Starkenstein and Weden (8) believe that there is great difference in potency between ferrous and ferric iron salts. It would seem that the dog can utilize the common forms of inorganic iron when there is need for iron.

Iron metabolism is admittedly a very difficult field where the hypotheses vary directly with the number of investigators. There is no certainty about the amount of food or inorganic iron which may be absorbed. Some workers claim that inorganic iron is not absorbed but only food iron utilized. There is much evidence to the contrary and the experiments cited below would seem adequate to show active participation on the part of inorganic iron by mouth in the dog. As iron is eliminated in the feces it may be difficult or impossible to say how much has been absorbed and eliminated or how much may pass inertly through the alimentary canal.

If we inquire how much iron is required to replace the iron withdrawn as hemoglobin by bleeding, we find an ample surplus in the standard ration. As fed the standard bread per 300 grams contains 20 mgm. iron as metal. The maintenance factor to replace the wear and tear of circulating red cells is an unknown but can scarcely amount to much even if we do not allow for conservation of the iron from the worn out red cells. For example, a 10 kilo dog will have a circulatory blood volume of about 800 cc. when anemic as described. Given a hemoglobin level of 50 per cent we calculate $8.00 \times 0.50 \times 13.8$ grams hemoglobin $= 55$ grams

hemoglobin in circulation. Estimating hemoglobin as containing 0.4 per cent iron as metal we find only 220 mgm. of iron in circulation. We can scarcely allow more than 10 mgm. of iron as the daily wastage by means of used up red cells. Probably the actual figure is only one-half or less and the body can carefully conserve iron when it is needed within the body.

Conservation of iron is touched upon in a comprehensive paper by Haldeman (3) who studies the bile pigments especially. Williamson and Ewing (14) claim that the iron reserve in liver and spleen in rats is increased by liver diet as well as by feeding iron citrate. In their last experiments iron analyses are not given and the storage might consist of hemoglobin building stones other than iron. Barkan (1) believes that the blood contains iron in addition to that fixed chemically as a part of the hemoglobin molecule. This amount is never great and its function a subject for speculation.

It would seem logical to conclude that there is need of but little iron for maintenance hemoglobin and a limited amount for the hemoglobin removed by bleeding. The positive effect of sizable doses of iron by mouth cannot be wholly to replace the iron lost by bleeding or as worn out red cells. We may designate this as a *salt effect* not unlike that observed after the feeding of balanced salt mixtures, copper salts or ash from tissues.

METHODS. Our general anemia program was outlined in the first papers of this series (12) and many important points there discussed need not be reviewed here. We stressed the importance of a *sustained maximal stimulus to hemoglobin production* in these dogs whose circulation hemoglobin level is maintained at 40 to 50 per cent hemoglobin, or approximately one-third normal for the dog. The surplus hemoglobin removed by bleeding to maintain this base line level is measured accurately and represents the capacity of the dog to form hemoglobin and red cells on the given food intake over and above the unknown red cell maintenance factor. These dogs are raised in our kennels and accustomed to the environment and experimental technique so that they lead normal, active lives undisturbed by the experimental observations, unless some note is made to the contrary. Uniformity of environment is very important and includes heat, ventilation, exercise and isolation to minimize cross infection.

It is important to remember that these standard dogs are in a condition of sustained anemia (40 to 50 per cent hemoglobin) month after month, year after year. Some of the dogs have been *continuously anemic* as indicated for 4 to 5 years and are reacting quantitatively to various specific diet intakes in the 4th year as they did in the 1st year. The experiments given in the tables are taken out of the dogs' experimental history which is continuous. The figures given represent the observations made at the *end of any given week* so that hemoglobin figures noted for the 1st week of

any feeding represent the production of hemoglobin due to 7 days' feeding of that diet as indicated.

The *reserve storage of hemoglobin factors* is another important point which bears on the interpretation of results. It is surprising to observe how difficult it may be to exhaust this emergency reserve in the healthy dog. We have made it a rule to produce our experimental anemia slowly over a 2 or 3 week period and to maintain this anemia for 2 or 3 months on the standard bread diet to insure the exhaustion of this reserve. During this period as much as 250 to 350 grams of hemoglobin may be removed by bleeding, representing a tremendous reserve which can scarcely be stored as finished red blood cells. This reserve is probably stored as intermediates which on demand can be combined to form the finished blood hemoglobin. After this period the dog will react with a small and constant hemoglobin output to the standard bread diet and will react uniformly to various diet factors as given in preceding papers.

The general methods used in these experiments have been described in detail in the first (12) and fifth (13) papers of this series. The *standard bread* used in all these experiments is described as to ingredients and preparation. This bread contains wheat flour, starch, bran, sugar, cod liver oil, canned tomatoes, canned salmon, yeast and a salt mixture. Bread (8) which contains a little salmon was used in all the experiments here recorded. This bread is a complete diet for an adult dog and will maintain a dog in health for long periods of time, if not indefinitely. This ration keeps the hemoglobin production at a very low level, the average being close to 1 to 3 grams of hemoglobin per week period over and above the unknown maintenance factor. There are individual differences and some dogs are constantly high in hemoglobin production on the basal rations as well as other diets. The *hemoglobin index* in these papers is a figure obtained by dividing the hemoglobin per cent by the red cell hematocrit in per cent. We believe this figure gives information of value as to the saturation of the red cell stroma with hemoglobin.

The salt mixture used in these experiments is that of McCollum and Simmonds, no. 185 without iron. Its constitution is as follows: sodium chloride 4.7 per cent, magnesium sulphate 7.2 per cent, acid sodium phosphate 9.4 per cent, basic potassium phosphate 25.8 per cent, calcium phosphate 14.6 per cent and calcium lactate 35.2 per cent. Wherever iron is added to this salt mixture it is noted in the tables. We note again that the standard bread used in these experiments contains 20 mgm. iron as metal per 300 grams bread as fed. Salmon contains but 1 mgm. iron per 100 grams as fed. It is obvious that these dogs are not suffering at any time from a shortage of iron intake if we can measure the needs for iron in terms of the iron incorporated in the newly manufactured hemoglobin. In all tables the iron salts used are designated but the figures in the tables indicate milligrams of iron as the metal.

Hemoglobin production due to any given diet period is calculated as follows. The hemoglobin level at the end of the fore period or last bread control week is compared with the hemoglobin level at the end of the after period. If these hemoglobin levels coincide within 2 or 3 per cent no correction is needed but if the figures are 3 or more per cent apart we correct for this difference in hemoglobin level. As calculated above, a 10 kilo dog has in circulation about 55 grams hemoglobin at a hemoglobin level of 50 per cent. Therefore we allow a gram of hemoglobin for each per cent difference of level. For example, a dog shows a 50 per cent hemoglobin level at the end of the fore period but in the after period is bled slightly too much which reduces his level to 43 per cent. Obviously we cannot attribute these 7 grams of hemoglobin to the given diet but to the overbleeding. We deduct these 7 grams from the tabulated figures to give a correct figure for hemoglobin production on the given diet. Experience shows that this calculation gives an accurate approximation of the actual findings when known amounts of hemoglobin are abstracted from these anemic dogs.

The total hemoglobin production due to any given diet is readily calculated from the tables. The control bread periods show 1.5 ± grams hemoglobin production each week. This amount of hemoglobin is removed for the routine determination of blood volume and hematocrit and some dogs will produce a little more than this amount of hemoglobin per week as the control level. The average value must be very close to 2 grams per week in our large colony. We deduct these 2 grams from each week's output above this figure and this includes the two weeks' feeding period plus the 2 or 3 weeks after control period. It has been amply proven (6) that on a favorable diet a dog stores substances which later during the unfavorable control diet or after period will be turned into hemoglobin and appear often in considerable amounts above the control level. This hemoglobin production of the after period obviously is due to the favorable diet or drug and is added to the hemoglobin production of the actual diet period. There is always more or less lag between the start of a favorable diet period and the rise in the curve of the hemoglobin production. That there is a lag in the fall of the hemoglobin production after any favorable diet period is not surprising. We may choose to explain this lag as due to the complicated internal metabolism of hemoglobin which must be built up from a variety of building stones present in the intestinal contents or stored in the body.

EXPERIMENTAL OBSERVATIONS. The experiments tabulated below illustrate a variety of reactions to iron in various amounts, alone and combined with other salts. These are only a few characteristic experiments out of a very large number of experiments which cannot be presented because of lack of space. Early in our work we observed that there was much more variation in hemoglobin production in our experiments with salts

than was observed in favorable meat product diet periods—for example, liver. Because of this conspicuous variation in the hemoglobin production in these salt experiments we have repeated these experiments over and over again using slightly different dosages and salt mixtures until we felt that we had come to a fairly clear understanding of these reactions. The

TABLE 161

Ferrous sulphate, ferric citrate and a salt mixture

DIET PERIODS 1 WEEK EACH	FOOD CONS.	WT.	PLASMA VOL.	RBC	COLOR INDEX	HB. INDEX	RBC HEMAT.	BLOOD HB. LEVEL	HB. REMOVED BLED
Food, grams per day	*per cent*	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*grams*
Dog 25–21 coach, male, adult									
Br. 350, salm. 100, Klim 25	100	16.7	844	5.0	0.48	2.21	21.7	48	1.5
† Fe 40 mgm. (ferrous)**	100	16.6	820	5.6	0.57	2.11	22.5	48	29.0
† Fe 40 mgm. (ferrous)**	100	16.7	857	5.5	0.51	2.19	20.5	45	17.1
Br. 350, salm. 100, Klim 25	100	16.8	830	5.5	0.49	2.27	17.7	40	16.0
Br. 350, salm. 100, Klim 25	100	17.1	888	4.7	0.48	2.24	20.2	45	1.5
Salt mixt.* 6 gm. no Fe**	100	17.0	850	5.3	0.50	2.31	19.6	45	12.7
Salt mixt.* 6 gm. no Fe**	83	16.5	868	5.2	0.42	2.22	20.0	44	1.4
Br. 375, salm. 100, Klim 25	96	17.1	900	5.1	0.47	2.16	22.1	48	1.5
Br. 375, salm. 100, Klim 25	99	16.9	856	6.3	0.40	2.05	21.7	45	11.3
Br. 375, salm. 100, Klim 25	96	17.2	890	5.3	0.37	2.04	19.1	39	1.2
†† Salt mixt.* 6 gm., Fe 38**	100	17.3	862	6.5	0.41	2.14	25.2	54	13.7
†† Salt mixt.* 6 gm., Fe 38**	100	17.7	830	7.0	0.44	2.17	21.4	47	25.2
Br. 375, salm. 100, Klim 25	100	17.7	824	5.4	0.44	2.24	21.1	47	1.4
Br. 375, salm. 100, Klim 25	98	17.8	858	5.2	0.47	2.10	22.2	42	9.6
Br. 375, salm. 100, Klim 25	83	17.5	1041	4.9	0.46	2.24	20.2	45	1.4

* McCollum and Simmonds, no. 185.

** Bread 350, salmon 100, Klim 25, daily diet.

† Iron given as ferrous sulfate.

†† Iron given as ferric citrate.

Iron in all tables recorded as milligrams of the metal.

$$\text{Hemoglobin index} = \frac{\text{Hemoglobin per cent}}{\text{Red cell hematocrit per cent}}.$$

following tables give illustrative reactions and by themselves might give little security but from our knowledge gained from over 40 similar experiments we have confidence in the few deductions which may be drawn from all these observations.

Table 161 shows the reaction of the anemic dog to a 40 mgm. daily dose of iron—both as ferrous sulphate and ferric citrate. As described under "methods," we calculate the hemoglobin production as about 29 grams hemoglobin per week due to ferrous sulphate and about 25 grams of hemoglobin per week due to ferric citrate. This is the optimum dosage of iron and ten times this dosage will scarcely raise this output level of hemoglobin. The balanced salt mixture in considerable amount causes only a slight increase in the hemoglobin production, in this experiment about 7

TABLE 162

Ferric chloride and sodium phosphate

DIET PERIODS 1 WEEK EACH	FOOD CONS.	WT.	PLASMA VOL.	RBC	COLOR INDEX	HB. INDEX	RBC HEMAT.	BLOOD HB. LEVEL	HB. REMOVED BLED
Food, grams per day	*per cent*	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*grams*
Dog 24–89 bull, female, adult									
Br. 300, salm. 100, Klim 25.	100					2.22	18.9	42	**0.6**
Fe 30 mgm.*	100					2.34	23.9	56	**13.3**
Fe 30 mgm.*	100					2.25	22.8	51	**24.1**
Fe 30 mgm.*	100	15.1	816	5.5	0.52	2.19	22.3	49	**24.1**
Fe 30 mgm.*	100					2.27	21.8	50	**13.8**
Fe 30 mgm.*	100					2.27	23.1	52	**24.8**
Fe 30 mgm.*	100					2.21	17.0	38	**23.1**
Br. 300, salm. 100, Klim 25.	100	15.5	936	3.7	0.59	2.19	19.9	44	**1.2**
Br. 300, salm. 100, Klim 25.	100					2.29	21.1	48	**0.9**
Sod. phosph. 4 grams*	100					2.31	19.0	44	**0.6**
Sod. phosph. 4 grams*	100					2.31	21.3	49	**0.7**
Br. 300, salm. 100, Klim 25.	100	13.3	858	5.8	0.45	2.19	20.7	45	**11.9**
Br. 300, salm. 100, Klim 25.	100					2.18	18.7	41	**0.6**

* Bread 300, salmon 100, Klim 25, daily diet.
Iron given as ferric chloride.

grams hemoglobin per week over and above the average bread control level. The addition of the salt mixture to the iron salt in the diet usually increases slightly the output due to the iron alone which may indicate the true level for the ferric citrate alone to be about 20 grams hemoglobin output per week. This reaction however is not always observed and at times the salt mixture appears to be almost inert (see table 167).

Table 162 gives the effect of ferric chloride in a smaller dose (30 mgm. iron as metal daily) continued over a long period—6 weeks in all. We note no increase or decrease of the iron effect as the usual 2-week period

is extended. There is but little carry over into the control week and that is indicated by a rise in the hemoglobin level from 38 to 44 per cent. We have tested all the various constituent salts which make up the salt mixture used in many of our experiments. All these salts given alone are relatively

TABLE 163

Ferric chloride, ferric citrate and a salt mixture

DIET PERIODS 1 WEEK EACH	FOOD CONS.	WT.	PLASMA VOL.	RBC	COLOR INDEX	HB. INDEX	RBC HEMAT.	BLOOD HB. LEVEL	HB. REMOVED BLED
Food, grams per day	*per cent*	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*grams*
Dog 24–25 bull, male, adult									
Br. 250, salm. 100, Klim 25	100	14.0	832	4.5	0.51	2.10	22.1	46	**1.4**
† Fe 25 mgm.**	100	14.7	825	3.8	0.58	2.22	19.6	44	**1.4**
† Fe 25 mgm.**	100	14.9	836	5.2	0.60	2.17	24.3	53	**30.8**
† Fe 25 mgm.**	100	14.2	798	4.6	0.50	2.16	21.1	46	**1.5**
† Fe 25 mgm.*	100	14.0	786	5.5	0.50	2.29	23.7	54	**16.2**
Br. 250, salm. 100, Klim 25	100	14.2	776	4.6	0.51	2.24	21.1	47	**1.4**
Br. 250, salm. 100, Klim 25	100	14.1	830	5.3	0.50	2.21	22.3	49	**14.4**
Br. 250, salm. 100, Klim 25	100	13.9	830	4.8	0.48	2.17	21.2	46	**1.4**
Br. 250, salm. 100, Klim 25	100	14.0	848	4.4	0.50	2.17	20.4	44	**1.4**
†† Fe 40 mgm.**	100	14.1	672	4.0	0.70	2.34	24.3	57	**19.3**
†† Fe 40 mgm.**	100	13.5	692	4.8	0.66	2.25	28.0	63	**37.5**
Br. 250, salm. 100, Klim 25	100	13.6	753	3.9	0.58	2.34	19.2	45	**1.2**
Br. 250, salm. 100, Klim 25	100	13.6	778	4.4	0.57	2.40	24.8	60	**17.4**
Br. 250, salm. 100, Klim 25	100	13.5	792	4.9	0.47	2.34	19.8	46	**1.4**
†† Fe 38, salt mixt.* 6 gm.**	100	13.3	780	5.8	0.53	2.40	26.0	62	**32.6**
†† Fe 38, salt mixt.* 6 gm.**	100	13.2	774	4.7	0.60	2.46	25.0	62	**20.6**
Br. 250, salm. 100, Klim 25	100	13.2	848	4.9	0.60	2.36	17.5	41	**32.6**
Br. 250, salm. 100, Klim 25	100	13.7	884	3.6	0.57	2.34	17.7	41	**1.2**

* McCollum and Simmonds, no. 185, without iron.

** Bread 250, salmon 100, Klim 25, daily diet.

† Iron given as ferric chloride.

†† Iron given as feric citrate.

or completely inert whereas the salt mixture usually shows some influence on hemoglobin regeneration, slight as it is. Sodium phosphate (4 grams daily) in this experiment at first sight seems to show a definite increase of 10 grams hemoglobin in the after period but we note that the hemoglobin level is 48 per cent at the end of the fore period and 41 per cent at the end

of the after period which will account for almost all of the 10 grams hemoglobin. We may say the reaction comes within the slight variations which are observed in control periods.

Table 163 gives us an opportunity to compare the optimum dose of ferric citrate (40 mgm. iron as metal) with a smaller dose of ferric chloride (25 mgm. iron as metal). The hemoglobin output on the optimum dose of

TABLE 164

Salt mixture with and without iron

DIET PERIODS 1 WEEK EACH	FOOD CONS.	WT.	PLASMA VOL.	RBC	COLOR INDEX	HB. INDEX	RBC HEMAT.	BLOOD HB. LEVEL	HB. REMOVED BLED
Food, grams per day	*per cent*	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*grams*
Dog 24–45 bull, female, adult									
Bread 500	100	13.9	1010					34	**1.3**
Salt. mixt.* 15 gm., no Fe**	94	13.3	885	5.2	0.49	2.04	25.0	51	**1.2**
Salt. mixt.* 15 gm., no Fe**	100	13.5	900	4.3	0.58	2.00	25.0	50	**1.2**
Bread 500	100	13.8	914	5.3	0.37	1.80	22.2	40	**17.7**
Milk 450, cream 100, br. 500	98	14.2	980	4.5	0.52	1.90	24.0	46	**1.0**
Milk 450, cream 100, br. 500	100	15.0	1030	5.2	0.43	1.97	22.6	45	**1.2**
Bread 500	100	15.0	1030	4.5	0.53	1.95	24.8	49	**12.2**
Bread 500	100	14.9	1024	5.6	0.40	1.92	23.3	45	**1.3**
Salt. mixt.* 15 gm., Fe 95**	100	15.1	1020	6.7	0.43	1.85	26.0	48	**16.5**
Salt. mixt.* 15 gm., Fe 95**	100	14.9	958	5.5	0.51	2.07	30.0	62	**17.0**
Bread 500	100	15.0	920	5.5	0.53	2.00	26.1	52	**16.9**
Bread 500	100	14.8	1000	5.8	0.46	2.10	25.0	52	**13.4**

* McCollum and Simmond, no. 185.

** Bread 500, daily diet.

Iron given as ferric citrate.

Iron in all tables recorded as milligrams of the metal.

$$\text{Hemoglobin index} = \frac{\text{Hemoglobin per cent}}{\text{Red cell hematocrit per cent}}.$$

ferric citrate is unusually large and amounts to about 34 grams per week while the smaller dose of ferric chloride is less potent and amounts to about 14 grams per week. The larger dose of ferric citrate plus the salt mixture in this dog gives an unusually large hemoglobin output of 42 grams hemoglobin per week which approximates the high output on liver or kidney feeding. However this unusual effect is not often observed in

combined iron and salt mixture feeding—compare the next table 164. We may say that this particular dog 24–25 reacts more vigorously to most diet factors and produces more hemoglobin per week than does the average dog. We have observed this peculiarity in a few dogs and it may persist for months and years or even through the entire life of the dog. Whether this indicates a larger mass of productive bone marrow we cannot say. It would seem reasonable that such variants might be found.

TABLE 165

Ferric citrate in large doses

DIET PERIODS 1 WEEK EACH	FOOD CONS.	WT.	PLASMA VOL.	RBC	COLOR INDEX	HB. INDEX	RBC HEMAT.	BLOOD HB. LEVEL	HB. REMOVED BLED
Food, grams per day	*per cent*	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*grams*
Dog 25–23, bull, male, adult									
Br. 300, salm. 100, Klim 25.	100	14.5	850	5.3	0.42	2.00	22.3	45	1.4
Fe 64 mgm.*	100	13.7	782	7.8	0.43	2.10	24.7	52	28.8
Fe 64 mgm.*	100	13.4	786	6.0	0.50	2.16	27.0	58	26.4
Fe 64 mgm.*	100	14.0	862	5.5	0.55	2.13	20.9	44	25.8
Fe 64 mgm.*	100	13.6	834	5.8	0.47	2.22	22.7	50	16.9
Br. 300, salm. 100, Klim 25.	100	14.2	832	6.1	0.43	2.04	22.6	46	16.8
Br. 300, salm. 100, Klim 25.	100	14.0	846	6.0	0.45	2.26	21.5	48	14.2
Br. 300, salm. 100, Klim 25.	100	13.8	888	5.0	0.43	2.11	20.2	43	1.2
Fe 400 mgm.*	100	13.7	828	6.3	0.53	2.17	22.1	48	32.3
Fe 400 mgm.*	100	14.1	830	7.3	0.42	2.13	25.0	53	16.3
Fe 400 mgm.*	100	13.9	770	7.0	0.44	2.29	20.5	47	31.9
Br. 325, salm. 100, Klim 25.	100	14.1	830	4.1	0.65	2.31	21.0	48	20.2
Br. 325, salm. 100, Klim 25.	100	14.3	838	4.2	0.49	2.13	19.3	41	1.2
Br 325, salm. 100, Klim 25.	100	14.3	867	4.9	0.46	2.11	21.5	45	1.3

* Bread 300, salmon 100, Klim 25, daily diet.
Iron given as ferric citrate.

Table 164 shows the influence of the balanced salt mixture without iron. The salt ration is rather heavy but this seems to exert little if any effect above a dose of one-half this amount or less. The hemoglobin produced amounts to about 10 grams per week. When we add iron to this salt ration we observe an output of about 31 grams hemoglobin per week above the control level. The iron intake is 95 mgm. metal per day which is more than double the optimum intake of 40 mgm. iron. The large excess iron intake over the optimum amount does not influence the hemoglobin output.

Large salt intake at times may cause a considerable shrinkage of the circulating plasma volume. A tendency in this direction is noted in table 164 where the lowest figures are seen in the salt feeding periods and the week immediately following. As a rule the blood plasma volumes in

TABLE 166

Ferrous ammonium sulphate, ferric chloride and sodium iodide

DIET PERIODS 1 WEEK EACH	FOOD CONS.	WT.	PLASMA VOL.	RBC	COLOR INDEX	HB. INDEX	RBC HEMAT.	BLOOD HB. LEVEL	HB. REMOVED BLED
Food, grams per day	*per cent*	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*grams*
Dog 24–26 bull, male, adult									
Br. 250, salm. 100, Klim 25	91	10.2	624	3.7	0.67	2.50	19.6	49	**1.4**
†Fe 30 mgm., NaI 300 mgm.*	100	10.2	644	3.8	0.61	2.42	19.1	46	**1.5**
†Fe 30 mgm., NaI 300 mgm.*	100	10.5	626	4.2	0.67	2.42	20.7	50	**14.5**
Br. 250, salm. 100, Klim 25	100	10.5	636	5.0	0.58	2.36	23.3	55	**14.7**
Br. 250, salm. 100, Klim 25	100	10.6	620	3.8	0.66	2.21	19.4	43	**10.1**
Br. 250, salm. 100, Klim 25	100	10.7	680	3.5	0.56	2.31	16.7	39	**1.0**
†Fe 30 mgm.*	100	10.5	634	4.1	0.60	2.34	21.1	49	**1.6**
†Fe 30 mgm.*	100	10.5	618	5.1	0.63	2.40	21.1	51	**27.8**
Br. 250, salm. 100, Klim 25	100	10.7	648	4.0	0.64	2.42	20.5	50	**9.8**
Br. 250, salm. 100, Klim 25	100	10.9	658	4.1	0.56	2.29	20.2	46	**1.3**
Br. 250, salm. 100, Klim 25	100	10.5	600	3.2	0.74	2.33	20.3	47	**1.3**
††Fe 42 mgm. (ferrous)*	100	10.5	626	4.7	0.62	2.36	26.6	63	**17.2**
††Fe 42 mgm. (ferrous)*	100	10.6	621	4.5	0.68	2.33	22.4	52	**30.5**
Br. 250, salm. 100, Klim 25	100	10.5	626	3.9	0.60	2.42	19.6	47	**2.3**
Br. 250, salm. 100, Klim 25	100	10.5	643	4.5	0.60	2.48	20.3	50	**17.6**
Br. 250, salm. 100, Klim 25	100	10.5	656	4.3	0.57	2.24	17.0	38	**11.9**
Br. 250, salm. 100, Klim 25	100	10.5	656	3.8	0.57	2.38	18.0	43	**1.4**

* Bread 250, salmon 100, Klim 25, daily diet.

† Iron given as ferric chloride.

†† Iron given as ferrous ammonium sulphate.

$$\text{Hemoglobin index} = \frac{\text{Hemoglobin per cent}}{\text{Red cell hematocrit per cent}}.$$

all these tables are quite constant for any given dog and indicate a uniformity of blood concentration which gives added security in the interpretation of the hemoglobin per cent levels. The hemoglobin index shows no unusual fluctuations.

Table 165 brings out an important point. Above the optimum intake of iron any large surplus has no effect in increasing the output of hemoglobin. This dog during a 3 week period received 400 mgm. iron as metal daily and put out 30 grams hemoglobin per week. This huge intake of iron is ten times the opimum dose yet the output is not increased over the amount to be expected for 40 mgm. iron daily. A dose of iron amounting to 64 mgm. iron as metal daily for a four week period shows a weekly output of hemoglobin of about 29 grams. We note no difference in weekly calculated average output whether the iron is given for 2, 3, 4 or 6 weeks. The iron reaction is uniform from week to week which perhaps is somewhat against the theory that iron acts as a stimulant or irritant to the bone marrow or hematopoietic organs. A true irritant usually shows some lessening in reaction as the exhibition of the drug is continued.

Table 165 also illustrates an interesting change in the hemoglobin index. This figure is obtained by dividing the hemoglobin per cent by the red cell hematocrit per cent. It may indicate the degree of saturation of the stroma matrix by the hemoglobin. In this experiment we note a tendency for this index to rise during heavy iron feeding. This may indicate that the hemoglobin is being formed a little more rapidly than the red cell stroma. This tendency is even more marked when iron and liver are fed together (7). These changes in the hemoglobin index are not constant but should be followed carefully in the hope that eventually one may get a lead as to the most important factors which go into the make up of the red cell stroma.

Table 166 illustrates several points. The optimum dose of iron given as ferrous ammonium sulphate (42 mgm. iron as metal daily) gives a high reaction of about 31 grams hemoglobin production each week. Evidently the body can assimilate and utilize for new hemoglobin production this particular iron salt. The reaction to a somewhat smaller daily dose of ferric chloride (30 mgm. as metal) is distinctly less and we observe a weekly output of about 22 mgm. hemoglobin.

The first experiment in table 166 shows the influence of a combined dose of iron and sodium iodide. Here we note less hemoglobin output (about 12 grams per week) than during a subsequent period of iron alone (about 22 grams hemoglobin per week). We have observed this in other experiments but it is not uniform and we hesitate to say that sodium iodide lessens the favorable effect of iron feeding. Yet this possibility must be kept in mind. We may refer also to other experiments with copper and iodide in the next paper and in an earlier publication (2).

Table 167 shows a low average reaction to a salt mixture minus iron which amounts to about 4 grams hemoglobin per week over and above the control bread level. This same salt mixture plus copper tartrate (40 mgm. daily as metal) shows an output of about 12 grams hemoglobin per week,

which modest increase we may assume is due to the copper. By contrast we note an enormous output of hemoglobin due to a moderate amount of ferric citrate which figures about 54 grams hemoglobin per week. This is

TABLE 167

Ferric citrate, copper tartrate and a salt mixture

DIET PERIODS 1 WEEK EACH	FOOD CONS.	WT.	PLASMA VOL.	RBC	COLOR INDEX	HB. INDEX	RBC HEMAT.	BLOOD HB. LEVEL	HB REMOVED BLED
Food, grams per day	*per cent*	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*grams*
Dog 24–45 bull, female, adult									
Bread 400, salm. 100.....	100	19.7	1140	4.7	0.46	2.21	19.3	43	1.4
Salt. mixt.* 6 gm. no Fe**	100	19.9	1240	4.8	0.43	2.22	18.6	41	1.2
Salt. mixt.* 6 gm. no Fe**	100	19.9	1206	4.9	0.41	2.22	18.0	40	1.2
Bread 400, salm. 100.....	100	19.9	1134	6.0	0.47	2.17	19.5	42	15.4
Bread 400, salm. 100.....	100	20.1	1234	5.2	0.51	2.21	17.0	38	1.0
Bread 350, salm. 100.....	100	18.7	1148	4.7	0.47	2.27	19.5	44	1.3
Fe 25 mgm.***.....	100	18.5	1014	3.1	0.85	2.36	25.7	61	15.1
Fe 25 mgm.***.....	100	18.4	932	4.6	0.64	2.38	24.9	59	33.9
Bread 350, salm. 100.....	100	18.3	1003	4.9	0.58	2.31	28.8	66	22.7
Bread 350, salm. 100.....	100	18.4	1064	5.2	0.53	2.36	22.5	53	30.3
Bread 350, salm. 100.....	100	18.3	1077	4.6	0.52	2.31	20.8	48	1.5
Bread 350, salm. 100.....	100	18.5	1138	5.3	0.51	2.42	21.7	52	18.7
Bread 350, salm. 100.....	100	18.1	1096	5.3	0.43	2.31	19.6	45	1.3
Salt mixt.* 6 gm., Cu 40***	100	18.2	1116	4.7	0.51	2.25	21.4	48	1.5
Salt mixt.* 6 gm., Cu 40***	100	18.5	1074	5.0	0.43	2.17	19.7	43	1.4
Bread 400, salm. 100.....	100	18.8	1102	5.4	0.46	2.44	23.8	58	14.6
Bread 400, salm. 100.....	100	19.2	1097	5.3	0.49	2.33	23.4	54	14.8
Bread 400, salm. 100.....	100	19.1	1104	4.8	0.49	2.25	20.9	47	1.4

* McCollum and Simmonds, no. 185.

** Bread 400, salmon 100, daily diet.

*** Bread 350, salmon 100, daily diet.

Iron given as ferric citrate.

Copper given as copper tartrate.

Copper and iron tabulated as milligrams of the metal.

equivalent to the standard reaction to heavy liver feeding. We note occasionally these very high output periods due to iron and again occasionally with standard iron intake a very low output level for hemoglobin. At present we cannot explain these wide fluctuations associated with iron

or metal salt intake but they contrast with the more uniform reaction noted with liver and kidney feeding. Possibly the unusual salt reactions are due in part to the storage of different intermediates due to slightly different experimental conditions in the preceding weeks.

There is one important point which should be mentioned here. This experiment (table 167) shows a large "carry over" into the after bread periods following the ferric citrate (25 mgm. as metal) by mouth. This is rather unusual and the total output extraordinarily high. *As a rule* iron therapy usually exerts all its effect during the weeks of iron intake and the first two weeks following. This rule holds whether we give iron for 2 weeks or 6 weeks. By contrast long periods of *liver feeding* as they are prolonged from 2 to 4 and 6 weeks show a largely increased carry over into subsequent weeks. We may observe that a storage has taken place during 4 weeks of feeding liver and it may require 4 to 6 weeks of control after period to exhaust this reserve. There is evidence that this reserve is held in the body in the form of intermediates of varied types which can be synthesized into hemoglobin as the emergency anemia demands continue.

Discussion. From these experiments it would appear that we cannot measure the iron needs of the body solely in terms of the iron required to build new hemoglobin whether for red cells or striated muscle. Apparently the body can utilize to advantage a considerable excess of inorganic as well as organic iron coming in through the intestinal tract. This optimum amount in these experiments is about 40 mgm. as metal in the form of iron salts plus 20 mgm. iron contained in the food daily intake which exceeds about three-fold the probable wastage and withdrawal of iron. For the sake of this comparison we may use 5 mgm. of iron as metal to cover the daily wastage in red blood cells and 17 mgm. of iron as metal to cover the average daily withdrawal by bleeding (table 161). As a matter of fact, this wastage of iron may be less than this because of the well recognized conservation within the body of iron coming from red cell disintegration.

One may inquire as to possible wastage of iron in the muscle hemoglobin molecule. This answer cannot be positive but the known facts would indicate a slow turnover and wastage of muscle hemoglobin in young dogs (9).

It is difficult to understand the mechanism by which inorganic salts may profoundly influence the production of blood hemoglobin in these experimental anemias. We may cite a few observations which bear on this question. Fasting dogs (10) usually produce more hemoglobin in anemia than dogs on a liberal carbohydrate intake which we believe indicates a careful conservation of intermediates derived from protein katabolism used for construction of new hemoglobin. By contrast we recall (6) that during a period of rapid gain in weight on a meat diet the anemic dog will

not form the expected amount of hemoglobin. Evidently material suitable for tissue building has been diverted from new hemoglobin construction. Probably there is a certain give and take within the body of essential amino acids and other materials suitable for tissue growth or repair as well as for new hemoglobin production. Possibly certain salts and metals influence this internal exchange of essential factors. We may imagine these salts as having some influence upon the direction of the flow of these building stones—now toward tissue growth or repair—now for body fluid protein maintenance—now for emergency new hemoglobin and red cell production. This is the picture which we have in mind when we use the term *salt effect.*

CONCLUSIONS

The optimum dose of iron by mouth in these experiments is about 40 mgm. iron as metal daily added to the basal ration iron. Above this level of intake a large excess of iron salts gives no further rise in the production of hemoglobin.

Iron has been given in the form of ferric chloride, ferric citrate, ferrous carbonate, ferrous sulphate and ferrous ammonium sulphate with similar results. The average weekly output of hemoglobin on the optimum iron salt intake is very close to 25 grams hemoglobin.

The basal ration of bread contains 20 mgm. of iron as metal per 300 grams bread as fed.

The optimum total intake of iron exceeds threefold the loss of iron by bleeding and wastage of red cells. It is obvious that this iron has some effect in the body other than that of mere replacement of iron in the lost or worn out hemoglobin.

This iron in excess of hemoglobin iron requirements obviously exerts some influence upon internal body metabolism so that *more hemoglobin is produced.* This may be designated as a *salt effect* and is probably similar to the effect noted with feeding salt mixtures, copper and other metals and ash from tissues.

Iron is the most potent metal so far tested in severe secondary anemia due to hemorrhage in dogs.

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BLOOD REGENERATION IN SEVERE ANEMIA

XVII. Influence of Manganese, Zinc, Copper, Aluminum, Iodine and Phosphates

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All these metals and salts are found in the body and some are known to take part in certain metabolic reactions. The interrelations of these substances are often complex and hard to define accurately as can be readily ascertained by a review of the conflicting views in the abundant contributions of recent years. Our interest in this field dates back to early experiments with apricot feeding in California which have been recently published (6). The inorganic ash of apricots, beef liver and pig kidney contains a potent salt mixture capable of bringing about a large increase of hemoglobin production (5). Analyses of these ashes showed a high content of iron, copper and aluminum as was found also in rats after salt feeding by Flinn and Inouye (2). There is no argument about iron and its positive effect in severe anemia due to hemorrhage in dogs but in nutritional anemias there are grounds for difference of opinion.

Copper has recently come into the limelight largely due to the work of the Wisconsin group (3). Copper is potent in nutritional anemia in rats but its effect in our anemic dogs is uncertain and without question it is far less potent than iron. Some of these experiments have been published (1) and others dealing with copper are tabulated below.

Manganese has come in for study and is known to be present in the livers of many domestic animals (4). Manganese has been studied in nutritional anemia in rats by Titus and Cave (8) who believe it to be potent and suggest that manganese may replace copper and supplement iron in new hemoglobin production. Our experiments with manganese show very irregular reactions, sometimes favorable for hemoglobin production and sometimes not. Manganese is toxic to dogs and must be administered with care.

Zinc has not been studied as much but is known to be present in domestic animals (4). It is not as potent as copper or manganese in our experiments and may in fact be almost inert under the conditions of these experiments.

Aluminum is well represented in the body and its metabolism has been

recently studied by Underhill and Peterman (9) who show that it is readily absorbed in the dog. Seibert and Wells (7) report extensive observations on rabbits to show that in these animals long continued administration of sodium aluminum sulphate by mouth will cause some anemia. It is possible that rabbits are especially sensitive to this material. In our experiments there is no evidence that aluminum influences hemoglobin production in severe anemia.

Sodium iodide can influence internal metabolism—for example the thyroid—but we can say nothing favorable about its effect upon hemoglobin regeneration. There is some evidence that this salt may actually inhibit the influence of iron or copper.

Artificial salt mixtures of considerable variety have been tested. The balanced salt mixture no. 185 of McCollum and Simmonds without iron gives the most favorable result. Mixtures made up of various salts minus iron have been made on the basis of the analyses of ashes of apricots, liver or kidney. These have so far been disappointing and we can report only negative results. From time to time we have tested the various individual salts which make up the McCollum and Simmonds salt mixture. Tables 175 and 176 show that the phosphates of calcium and potassium have little if any effect upon hemoglobin regeneration.

Various mixtures of copper, manganese and zinc with and without iron are tested in experiments given below. As a rule these mixtures are less potent than one might expect from the known potency of the individual metals. When we give liver plus iron we expect to get the *sum* of the individual effects and note a large output of hemoglobin in anemia. When we give copper or manganese plus iron we rarely observe an output of hemoglobin above that to be expected from the iron alone. There is little if any summation of these effects due to metals in our experiments.

Experimental observations. The details of method are given in the preceding paper. The salts in these experiments were mixed with the food and eaten without difficulty. All salts are given as indicated in the tables but are recorded as milligrams of the metal except sodium iodide and the phosphates recorded as salt. The hemoglobin production per week is calculated exactly as outlined in the preceding paper.

Table 171 gives two satisfactory experiments with manganous chloride with and without iron. Manganese by mouth is toxic to anemic dogs and must be used with care. These dogs were not disturbed by a good sized daily intake of manganese—40 to 43 mgm. per day, figured as the metal. The food intake and weight curve were not disturbed. The first dog (23–1, table 171) shows a weekly output of about 15 grams hemoglobin above the control level on a daily intake of manganese 40 mgm. and iron 20 mgm. We might expect this same output of hemoglobin from the same dose of iron alone and see no evidence that the manganese has supplemented the iron salt effect.

The second dog (24–45, table 171) shows an unusually favorable reaction to manganese alone and a weekly output of about 19 grams hemoglobin over the control period. When this manganese dosage (43 mgm.) is supplemented with iron (27 mgm.) we record only about 26 grams hemo-

TABLE 171

Manganese alone and combined with iron

DIET PERIODS 1 WEEK EACH	FOOD CONS.	WT.	PLASMA VOL.	RBC	COLOR INDEX	HB. INDEX	RBC HEMAT.	BLOOD HB. LEVEL	HB. REMOVED BLED
Food, grams per day	*per cent*	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*grams*
Dog 23–1 bull, male, adult									
Br. 375, salm. 100, Klim 25	100	16.3	931	5.0	0.47	2.25	21.0	47	**1.4**
Br. 375, salm. 100, Klim 25	100	16.3	973	5.2	0.42	2.29	19.3	44	**1.3**
† Fe 20 mgm., Mn. 40*	100	16.2	964	5.3	0.47	2.42	22.2	54	**12.2**
† Fe 20 mgm., Mn. 40*	100	16.9	966	5.0	0.47	2.24	21.0	47	**1.4**
Br. 375, salm. 100, Klim 25	100	16.9	950	6.0	0.48	2.31	21.2	49	**22.8**
Br. 375, salm. 100, Klim 25	100	16.9	1024	4.3	0.49	2.24	18.6	42	**1.2**
Dog 24–45 bull, female, adult									
Br. 400, salm. 100	100	21.4	1172	4.5	0.53	2.36	20.4	48	**1.6**
++ Mn. 43 mgm., Fe 27**	100	21.5	1146	4.6	0.51	2.29	20.4	47	**1.5**
++ Mn. 43 mgm., Fe 27**	100	21.6	1094	5.4	0.55	2.58	28.4	73	**27.4**
Br. 400, salm. 100	100	21.5	1100	5.3	0.61	2.36	22.9	54	**29.3**
Br. 400, salm. 100	100	21.7	1220	4.0	0.59	2.40	19.7	47	**1.4**
Mn. 43 mgm.**	100	21.0	1280	5.7	0.53	2.40	25.4	60	**25.5**
Mn. 43 mgm.**	100	20.6	1098	4.6	0.55	2.17	20.0	44	**12.9**
Br. 400, salm. 75	100	20.6	1177	4.8	0.55	2.27	18.0	41	**12.9**
Br. 400, salm. 75	100	20.5	1240	3.9	0.55	2.21	19.6	43	**1.3**

* Bread 375, salmon 100, Klim 25, daily diet.

** Bread 400, salmon 100, daily diet.

† { Manganese given as manganous chloride.
  { Iron given as ferric chloride.

++ Iron given as ferric citrate.

Iron and manganese recorded as milligrams of the metal.

globin as the weekly output. This output is scarcely above the level to be expected from the iron alone. Although alone the manganous chloride was potent in this experiment there was no evidence for summation when the two metals were administered together. The same lack of summation is noted when copper and manganese are combined (table 172).

Table 172 illustrates the irregularity of reaction which may be noted with these metals. The first dog 25–97 shows a distinct reaction to a small dose of manganese—about 12 grams hemoglobin per week above control level, which is a fair salt reaction. When a larger dose of manganese is

TABLE 172

Manganese alone and combined with copper and sodium iodide

DIET PERIODS 1 WEEK EACH	FOOD CONS.	WT.	PLASMA VOL.	RBC	COLOR INDEX	HB. INDEX	RBC HEMAT.	BLOOD HB. LEVEL	HB. REMOVED BLED
Food, grams per day	*per cent*	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*grams*
Dog 25–97 bull, male, adult									
Br. 450, salm. 100, Klim 25	100	17.7	1070	4.7	0.47	2.19	20.0	44	1.3
Br. 450, salm. 100, Klim 25	100	17.7	1070	5.3	0.43	2.19	21.2	46	1.4
Mn, 7.5 mgm.**	100	17.7	1041	4.9	0.49	2.22	21.4	48	1.5
Mn. 7.5 mgm.**	100	18.0	1075	5.9	0.44	2.25	20.7	47	14.6
Br. 450, salm. 100, Klim 25	100	17.6	1080	4.9	0.48	2.22	21.0	47	1.4
Br. 450, salm. 100, Klim 25	85	17.0	1080	5.3	0.50	2.27	19.9	45	14.9
Br. 400, salm. 100, Klim 25	88	17.1	1033	5.4	0.41	2.14	20.7	44	1.4
Mn. 36 mgm.***	100	17.2	1055	5.8	0.41	2.14	22.2	48	1.4
Mn. 36 mgm.***	100	16.9	1109	5 2	0.42	2.14	20.3	44	1.3
Br. 375, salm. 100, Klim 25	100	17.0	1096	5.3	0.43	2.07	22.4	46	1.4
Dog 24–59 bull, male, adult									
Bread 450, salm. 75	100	16.3	988	4.4	0.52	2.24	20.6	46	1.5
Mn. 40, Cu 21, NaI 100*	100	16.2	999	5.1	0.50	2.36	19.6	46	11.9
Mn. 40, Cu 21, NaI 100*	100	16.1	990	4.4	0.50	2.27	19.4	44	1.3
Bread 450, salm. 75	100	16.1	978	4.7	0.49	2.29	20.1	46	1.4
Bread 450, salm. 75	100	16.2	1029	5.2	0.44	2.21	20.8	46	1.4

* Bread 450, salmon 75, daily diet.

** Bread 450, salmon 100, Klim 25, daily diet.

*** Bread 375, salmon 100, Klim 25, daily diet.

Manganese given as manganous chloride.

Copper given as copper sulphate.

Sodium iodide recorded as milligrams of the salt.

Iron and manganese recorded as milligrams of the metal.

given after a control period we see not the slightest increase in hemoglobin output. There is no loss of weight and no lack of appetite. This negative reaction is to be contrasted with the distinctly favorable one in table 171—the same dosage of manganese being employed.

The second experiment (table 172—dog 24-59) shows the effect of average doses of manganous chloride, copper sulphate and sodium iodide. There is only a very moderate effect and an increased hemoglobin output of only 5 grams per week. One might expect more hemoglobin output from the copper salt alone. Not only is there no summation of effects

TABLE 173

Zinc alone and combined with iron

DIET PERIODS 1 WEEK EACH	FOOD CONS.	WT.	PLASMA VOL.	RBC	COLOR INDEX	HB. INDEX	RBC HEMAT.	BLOOD HB. LEVEL	HB. REMOVED BLED
Food, grams per day	*per cent*	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*grams*
Dog 24-25 bull, male, adult									
Br. 300, salm. 100, Klim 25	100	13.0	790	4.6	0.52	2.27	21.3	48	**1.4**
Zinc 2.7 mgm.*	100	13.3	816	4.4	0.51	2.21	20.6	45	**1.4**
Zinc 2.7 mgm.*	100	13.2	796	4.9	0.51	2.33	21.4	50	**1.5**
Br. 300, salm. 100, Klim 25	100	13.3	803	5.5	0.51	2.33	19.5	45	**13.7**
Br. 300, salm. 100, Klim 25	100	13.3	855	4.4	0.52	2.22	20.5	46	**1.3**
Zinc 27 mgm.*	100	13.2	812	4.6	0.53	2.21	22.1	49	**1.4**
Zinc 27 mgm.*	100	13.7	866	4.4	0.49	2.19	19.5	43	**1.2**
Br. 300, salm. 100, Klim 25	100	13.2	848	4.5	0.48	2.22	19.3	43	**1.3**
Br. 300, salm. 100, Klim 25	100	13.4	810	5.0	0.51	2.22	18.8	42	**11.1**
Br. 300, salm. 100, Klim 25	100	13.5	882	4.2	0.45	2.21	17.1	38	**1.1**
Zinc 27 mgm., Fe 30*	100	13.2	812	5.7	0.46	2.36	21.4	51	**11.2**
Zinc 27 mgm., Fe 30*	100	13.6	848	5.6	0.52	2.38	21.3	51	**25.1**
Br. 300, salm. 100, Klim 25	100	13.8	890	5.2	0.51	2.21	20.3	45	**12.7**
Br. 300, salm. 100, Klim 25	100	13.7	840	5.1	0.47	2.33	20.8	48	**1.3**

* Bread 300, salmon 100, Klim 25, daily diet.

Zinc given as zinc acetate
Iron given as ferric citrate } but tabulated as milligrams of the metal.

$$\text{Hemoglobin index} = \frac{\text{Hemoglobin per cent}}{\text{Red cell hematocrit per cent}}.$$

but there is a hint that possibly the sodium iodide has inhibited slightly the copper effect—compare table 166 in the preceding paper for a similar reaction due to iron plus sodium iodide.

Table 173 gives some information about zinc acetate. A very small dose of zinc causes a trivial rise in the output of hemoglobin which is almost within the limits of physiological fluctuation on the standard bread

ration. A larger dose of zinc causes no increase in the hemoglobin output. There is an apparent increase which actually is to be explained by an excess bleeding as is seen by comparing the hemoglobin levels before (46 per cent) and at the end of the after period (38 per cent). When we correct for this

TABLE 174

Aluminum, antimony and sodium iodide

DIET PERIODS 1 WEEK EACH	FOOD CONS.	WT.	PLASMA VOL.	RBC	COLOR INDEX	HB. INDEX	RBC HEMAT.	BLOOD HB. LEVEL	HB. REMOVED BLED
Food, grams per day	*per cent*	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*grams*
Dog 24–22 coach, female, adult									
Br. 325, salm. 100, Klim 25	92	13.1	746	4.4	0.47	1.99	21.0	42	1.2
Aluminum 70 mgm.*	91	12.9	770	4.4	0.49	2.03	21.1	43	1.3
Aluminum 60 mgm.*	85	12.9	792	5.2	0.45	2.03	23.2	47	1.3
Br. 300, salm. 100, Klim 25	94	13.0	785	4.8	0.47	2.02	22.4	45	1.3
Br. 300, salm. 100, Klim 25	100	13.1	782	4.9	0.42	2.11	19.6	41	1.3
Dog 24–2 bull, male, adult									
Bread 450, salm. 75	100	17.0	1012	5.3	0.46	2.11	22.6	48	1.5
Antimony 0.9 mgm.**	100	16.8	933	5.0	0.47	2.16	21.9	47	1.4
Antimony 0.9 mgm.**	100	16.9	939	5.8	0.44	2.06	23.8	49	1.4
Bread 450, salm. 75	97	16.9	966	5.8	0.47	2.14	20.0	43	14.5
Bread 450, salm. 75	95	16.8	947	5.4	0.44	2.19	21.5	47	1.5
Dog 23–3 bull, female, adult									
Bread 325, salm. 100	98	14.3	762	3.4	0.59	2.31	17.3	40	1.2
NaI 600 mgm.***	80	14.1	723	4.0	0.62	2.29	17.4	40	12.4
NaI 500 mgm.***	97	14.4	800	3.1	0.65	2.27	17.6	40	1.2
Bread 325, salm. 100	91	14.3	689	4.3	0.50	2.21	19.6	43	1.2
Bread 325, salm. 100	93	14.3	770	4.5	0.52	2.19	21.3	47	1.5

* Bread 300, salmon 100, Klim 25, daily diet. ** Bread 450, salmon 75, daily diet. *** Bread 325, salmon 100, daily diet.

Aluminum given as aluminum sodium sulphate, antimony given as antimony chloride, and tabulated as milligrams of the metal. Sodium iodide tabulated as salt.

it absorbs about all of the tabulated 11 grams of hemoglobin removed by bleeding.

Zinc plus iron shows a good production of hemoglobin which amounts to

about 26 grams per week over the control periods. This dose of iron alone could account for all of this hemoglobin output. We may refer to an experiment published elsewhere (1) which shows the same reaction to zinc chloride plus copper tartrate. Only the reaction to be expected from the copper alone was recorded. In our experiments evidently zinc comes very close to being absolutely inert.

Table 174 shows no evidence that aluminum or antimony salts in small doses have any conspicuous effect upon hemoglobin production in these

TABLE 175

Acid potassium phosphate, copper, iron and sodium iodide

DIET PERIODS 1 WEEK EACH	FOOD CONS.	WT.	PLASMA VOL.	RBC	COLOR INDEX	HB. INDEX	RBC HEMAT.	BLOOD HB. LEVEL	HB. REMOVED BLED
Food, grams per day	*per cent*	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*grams*
Dog 25–21 coach, male, adult									
Br. 300, salm. 75, Klim 25	100	15.2	854	5.8	0.41	1.93	24.8	48	**1.4**
Br. 300, salm. 75, Klim 25	100	15.1	803	5.7	0.42	1.99	24.0	48	**1.4**
Acid potass. phosph. 1 gm.*	100	15.1	824	6.4	0.34	2.00	21.8	44	**1.3**
Acid potass. phosph. 1 gm.*	100	15.1	941	6.3	0.41	2.13	19.6	42	**10.8**
Br. 350, salm. 75, Klim 25	100	15.3	814	6.3	0.39	2.08	23.4	49	**1.5**
Br. 300, salm. 100, Klim 25	100	17.6	914	4.7	0.49	2.21	20.8	46	**1.4**
Br. 300, salm. 100, Klim 25	100	17.6	891	4.5	0.52	2.29	20.3	47	**1.5**
NaI 100, Fe 20, Cu 21** ...	100	17.6	858	5.8	0.50	2.24	23.5	53	**19.1**
NaI 100, Fe 20, Cu 21** ...	100	17.7	840	6.2	0.51	2.24	18.0	40	**23.3**
Br. 325, salm. 100, Klim 25	100	17.6	869	4.6	0.53	2.19	18.0	39	**9.1**
Br. 325, salm. 100, Klim 25	100	17.8	888	4.2	0.49	2.14	19.0	41	**1.3**

* Bread 350, salmon 75, Klim 25.

** Bread 300, salmon 100, Klim 25.

Iron given as ferric chloride
Copper given as copper sulphate
} and tabulated as milligrams of the metal.

Sodium iodide and phosphate tabulated as salt.

experiments. The experiment with aluminum is frankly negative and the experiment with antimony shows a trivial increase in the after period amounting to about 6 grams hemoglobin per week above the control level. When sodium iodide is given alone in the standard diet there may be a slight increase in hemoglobin production which amounts to about 8 grams per week (table 174, dog 23-3). We note elsewhere that on occasions sodium iodide given with copper or iron salts may appear to inhibit slightly the expected favorable effect from copper or iron alone.

Table 175 shows that a liberal intake of acid potassium phosphate in the diet (1 gram daily) has little if any influence upon hemoglobin regeneration. As figured there is an increase of about 5 grams hemoglobin per week above the control level. The second experiment shows the influence of copper, iron and sodium iodide in average dosage. We calculate an output of about 19 grams hemoglobin per week above the control level which is scarcely more than would be expected from the iron alone. Even

TABLE 176

Calcium phosphate and potassium phosphate

DIET PERIODS 1 WEEK EACH	FOOD CONS.	WT.	PLASMA VOL.	RBC	COLOR INDEX	HB. INDEX	RBC HEMAT.	BLOOD HB. LEVEL	HB. REMOVED BLED
Food, grams per day	*per cent*	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*grams*
Dog 23–1 bull, male, adult									
Br. 375, salm. 100, Klim 25	100	15.9	946	4.5	0.52	2.42	19.3	47	**1.4**
Calc. phosph. 4 grams*....	100	15.5	954	4.8	0.51	2.27	21.6	49	**1.4**
Calc. phosph. 4 grams*....	100	15.7	996	4.4	0.48	2.34	18.1	42	**1.3**
Br. 375, salm. 100, Klim 25	100	15.8	1020	4.4	0.55	2.42	19.9	48	**1.4**
Br. 375, salm. 100, Klim 25	100	16.2	1042	5.0	0.41	2.27	18.1	41	**1.2**
Dog 23–3 bull, female, adult									
Br. 350, salm. 100, Klim 25	100	14.4	758	3.4	0.65	2.34	18.7	44	**1.5**
Potass. phosph. 2 grams**	98	14.6	766	4.2	0.46	2.27	17.0	39	**1.2**
Potass. phosph. 2 grams**	93	14.5	722	4.4	0.65	2.36	17.0	40	**13.3**
Br. 350, salm. 100, Klim 25	75	14.4	748	3.9	0.55	2.40	17.8	43	**1.2**
Br. 275, salm. 100, Klim 25	92	14.5	734	4.0	0.56	2.29	19.6	45	**1.3**

* Bread 375, salm. 100, Klim 25, daily diet.

** Bread 350, salm. 100, Klim 25, daily diet.

$$\text{Hemoglobin index} = \frac{\text{Hemoglobin per cent}}{\text{Red cell hematocrit per cent}}.$$

Calcium phosphate and potassium phosphate tabulated as salt.

copper alone might cause as much increase in hemoglobin output but this would be less certain than with iron. There is no evidence of summation of these effects of the two salts. In this type of mixture sodium iodide may at times appear to exert some inhibition on the hemoglobin production.

Table 176 shows the influence of two salts of the McCollum and Simmonds salt mixture. Calcium phosphate in fairly large doses (4 grams daily) causes no increase in the hemoglobin output. In fact there is

actually a drop in the hemoglobin level during the feeding and after periods. We may explain a part of this decrease in hemoglobin level to a gradual increase in the plasma volume as shown in the table but there may be other factors such as actual inhibition of hemoglobin production. This last possibility is extremely difficult to establish.

In the second experiment (table 176) we note a larger dosage of potassium phosphate (compare table 175) which likewise has very little influence on the hemoglobin regeneration. We calculate an increase of about 6 grams hemoglobin per week above control levels.

These experiments as a rule show no wide fluctuations in blood plasma volumes. Rapid changes may be observed frequently with abundant salt intake and if not controlled by blood volume determinations may lead one to erroneous conclusions as to the level of hemoglobin production. In the face of these very irregular reactions to these heavy metals it is absolutely necessary to have the assurance that the picture is not further confused by wide fluctuation in blood volume. We note an interesting contrast in blood plasma volumes in the two dogs in table 176. The first dog (23–1) shows a normal plasma volume of 65 cc. per kilo and this dog is active and in good average condition. The second dog (23–3) shows a subnormal plasma volume of 51 cc. per kilo. This dog is over-weight, quite fat and sluggish.

CONCLUSIONS

Manganese by mouth causes very irregular responses—sometimes favorable for hemoglobin regeneration, sometimes not.

Manganese is probably somewhat less potent than *copper* salts which also are uncertain in their reaction in this type of experiment.

Zinc in our experiments shows reactions which are practically negative.

Iron salts in various combinations with manganese, copper or zinc, give hemoglobin production levels almost exactly similar to the production expected from the iron alone. There is no evidence for summation of these effects.

Aluminum and antimony in the dosage employed show no evidence of a potent effect.

Potassium and calcium phosphates have little if any influence upon hemoglobin regeneration.

Sodium iodide is to be classed as almost inert and it may even at times inhibit somewhat the salt effect of iron or copper.

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BLOOD REGENERATION IN SEVERE ANEMIA[1]

XVIII. Influence of Liver and Blood Sausage, Veal, Eggs, Chicken and Gelatin

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Meat products vary greatly in their potency for hemoglobin production in the anemic dog. From time to time we have reported experiments to show the potency of various meat products. Liver stands at the head of the list (3) followed closely by kidney (4). Pancreas, bone marrow and spleen (7) are moderately favorable for hemoglobin regeneration but average less than half the potency of liver. Skeletal, heart and smooth muscle have been studied and it is somewhat surprising to note that of these three skeletal muscle is least potent and smooth muscle most potent. Heart muscle is intermediate in its content of these factors which favor new hemoglobin production.

Smooth muscle (6) varies a good deal in its potency depending upon its source and possibly other factors, for example the adherent mucous membranes. Beef stomach in liberal feeding was less potent than the pregnant sow's uterus. The mucous membranes are complicating factors. However we note a remarkable reaction to the feeding of chicken gizzard—almost the equal of liver feeding in promoting hemoglobin regeneration. In all these three tissues smooth muscle is an important constituent—particularly so in the gizzard where the epithelium is very scanty and the smooth muscle rich in muscle hemoglobin. These gizzard muscles do a vast amount of work and must require liberal replacement factors. Probably substances are stored in these muscle fibres, which the body can use to fabricate new hemoglobin and red cells.

These experiments with smooth muscle tissue including the beef stomach have an immediate interest in relation to recent work in human anemia. Sturgis and Isaacs (5) have recently reported a favorable effect from the

[1] This work has been aided by a National Live Stock and Meat Board Fellowship of the National Research Council. We are indebted to the Rochester Packing Company for a liberal supply of certain meat products. We acknowledge with pleasure the friendly advice and assistance of Dr. E. B. Forbes and Dr. C. Robert Moulton.

use of pig stomach in human pernicious anemia with a remission similar to that produced by liver feeding. This work followed logically from the interesting observations of Castle (1), who showed that beef muscle digested in the normal human stomach gave a product which could effect a remission in pernicious anemia. One explanation obviously is that the normal stomach produces something which can bring about this remission in pernicious anemia. The feeding of pig stomach and the production of a prompt remission in pernicious anemia would seem to favor this explanation but we should not forget that smooth muscle is potent in experimental anemia and may be potent in pernicious anemia. It is possible that both the smooth muscle and the mucous membrane may be concerned.

The meat products reviewed in this paper present wide differences in potency as measured by new hemoglobin and red cell production. The liver and blood sausage feeding is quite effective and the output of hemoglobin amounts to about one-third to one-half that observed with whole liver feeding. Veal and chicken are less potent and correspond closely to beef skeletal or beef heart muscle. Gelatin, an incomplete protein, is of particular interest. It is scarcely as potent as heart muscle or veal. These experiments are inconclusive as to whether tryptophane or tyrosine are needed in new hemoglobin building as the experiments cannot be extended over long periods of time due to feeding difficulties. Moreover gelatin alone is not tolerated for any considerable time and the standard bread may supply sufficient tyrosine and tryptophane. Evidently gelatin adds something other than tryptophane or tryosine to the standard bread control diet which enables the body to fabricate more hemoglobin.

EXPERIMENTAL OBSERVATIONS. The various methods are described in paper XVI. There were no feeding difficulties with any of these meat products except gelatin. Gelatin was cooked with sufficient water to give a firm jelly on cooling. This was incorporated in the bread mixture and in the experiments tabulated was completely eaten. Many dogs will not eat much gelatin beyond a period of a few days. The weights given in the tables for gelatin refer to the weights of the dry thin sheets of gelatin as purchased. The hemoglobin output per week is calculated as described in paper XVI.

Table 181 shows some variety in the total hemoglobin output per week of liver sausage feeding. The lowest output is about 19 grams hemoglobin per week (dog 24–49) on an intake of 200 grams per day. The highest output is about 44 grams hemoglobin per week (dog 24–45) on an intake of 300 grams per day. This second dog uniformly puts out a good deal more hemoglobin on any given diet than the average figures. This is a permanent and personal peculiarity of this dog. The third experiment (dog 25–46) shows more nearly average values for hemoglobin output (25 grams per week) on an intake of 200 grams liver sausage daily.

Liver sausage contains varying amounts of material other than liver—for example, meat scraps, dried milk and cracker meal or flour. These materials compared with whole liver are relatively inert and any hemo-

TABLE 181

Liver sausage

DIET PERIODS 1 WEEK EACH	FOOD CONS.	WT.	PLASMA VOL.	RBC	COLOR INDEX	HB. INDEX	RBC HEMAT.	BLOOD HB. LEVEL	HB. REMOVED BLED
Food, grams per day	*per cent*	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*grams*
Dog 24–49 Bull, female, adult									
Bread 400, salm. 75.....	72	16.1	961	4.5	0.43	1.79	21.7	39	1.1
Liver sausage 200, br. 300..	100	16.7	1060	5.2	0.49	1.90	25.2	48	14.7
Liver sausage 200, br. 300..	97	17.0	1065	4.9	0.47	1.89	24.3	46	1.4
Bread 350, Klim 25*.....	75	15.9	964	5.4	0.52	1.96	23.2	45	18.0
Bread 350, Klim 25*.....	100	16.0	952	4.6	0.53	1.91	25.7	49	1.6
Dog 24–45 Bull, female, adult									
Bread 450, salm. 50.....	100	18.7	1170	3.7	0.47	2.04	17.0	35	1.0
Liver sausage 300, br. 300.	100	20.0	1175	4.8	0.43	2.10	19.7	41	1.5
Liver sausage 300, br. 200.	100	20.5	1050	5.5	0.63	2.11	24.5	52	35.6
Bread 450, salm. 50.....	100	20.8	1155	4.9	0.51	2.10	23.7	50	1.6
Bread 450, salm. 50.....	100	21.0	1150	4.5	0.61	2.25	25.2	57	18.9
Bread 450, salm. 50.....	100	21.2	1115	5.1	0.57	2.03	24.5	50	15.6
Bread 450, salm. 50.....	100	21.2	1285	5.0	0.55	2.08	27.1	57	18.4
Bread 450, salm. 50.....	100	20.9	1177	4.5	0.53	2.00	23.8	48	1.4
Dog 25–46 Bull, female, young adult									
Br. 275, salm. 100, Klim 25.	90	10.6	670	5.3	0.40	1.69	25.4	43	1.2
Br. 275, salm. 100, Klim 25.	92	10.7	584	6.1	0.38	1.74	26.9	47	1.4
Liver sausage 200, br. 200..	100	10.9	590	6.6	0.53	1.88	28.8	54	34.0
Liver sausage 200, br. 200..	100	11.1	542	5.6	0.52	1.86	20.8	39	25.9
Bread 275, salm. 100.....	100	10.9	661	4.1	0.49	1.91	21.1	40	1.2
Bread 275, salm. 100.....	100	10.8	645	4.6	0.45	1.86	22.2	41	1.3

* Meat scraps 75 grams added to daily diet.

globin reaction observed after liver sausage feeding must be attributed largely to the liver content. The sausage used in these experiments was purchased from the Rochester Packing Company and was known to contain 21 per cent of whole pig liver. There is no evidence that the process of

manufacture destroys any of this potency for hemoglobin production inherent in the fresh or boiled liver. The hemoglobin and red cell output depends upon the percentage of liver contained in the liver sausage and

TABLE 182

Blood sausage

DIET PERIODS 1 WEEK EACH	FOOD CONS.	WT.	PLASMA VOL.	RBC	COLOR INDEX	HB. INDEX	RBC HEMAT.	BLOOD HB. LEVEL	HB. REMOVED BLED
Food, grams per day	*per cent*	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*grams*
Dog 25–46 Bull, female, young adult									
Bread 375, salm. 75	100	10.0	606	5.5	0.39	1.96	21.9	43	1.3
Blood sausage 200, br. 250	100	10.4	538	7.5	0.38	1.78	23.2	41	16.6
Blood sausage 200, br. 250	100	11.0	587			1.96	25.0	49	1.4
Bread 375, salm. 75	100	10.9	571	6.0	0.47	1.90	21.4	41	12.2
Bread 425, salm. 75	96	10.9	606	5.1	0.46	2.00	23.6	47	1.2
Dog 24–49 Bull, female, adult									
Bread 450	100	15.9	961	4.4	0.50	2.19	20.3	44	1.4
Blood sausage 300, br. 300	98	16.7	997	5.4	0.53	2.17	23.2	50	19.4
Blood sausage 300, br. 300	80	16.7	926	6.0	0.46	2.13	23.4	50	20.2
Bread 450, salm. 75	61	16.3	878	5.4	0.47	1.94	19.9	39	14.7
Bread 450, salm. 100	99	16.4	962	4.5	0.52	1.97	23.8	47	1.4
Dog 24–45 Bull, female, adult									
Bread 450	100	17.0	1080	5.5	0.44	2.10	23.0	48	1.4
Blood sausage 400, br. 200	100	18.4	1064	5.1	0.47	2.11	26.0	55	21.0
Blood sausage 400, br. 200	100	19.6	1043	5.2	0.53	1.94	26.2	57	15.7
Bread 500	100	19.2	1078	5.4	0.51	2.00	24.1	48	18.5
Bread 500	100	19.2	1126	5.8	0.45	1.94	21.2	41	20.2
Bread 550	100	19.3	1120	4.5	0.61	2.05	24.5	50	18.9
Bread 550	100	19.0	988	4.3	0.51	2.05	21.2	44	1.4

$$\text{Hemoglobin index} = \frac{\text{Hemoglobin per cent}}{\text{Red cell hematocrit per cent}}.$$

falls considerably below the whole liver effect due to the admixture of relatively inert food material in the standard process of sausage manufacture.

Table 182 shows that blood sausage contains much material which is

potent for the production of new hemoglobin and red cells. The hemoglobin contained within the sausage is responsible for much of this favorable reaction and the new produced hemoglobin can be roughly calculated on that basis. We have shown (8) that fresh hemoglobin taken into the stomach will contribute about 10 per cent of the intake to new hemoglobin production—that is, an intake of 200 grams hemoglobin per week will effect an average increase in hemoglobin output of 20 grams above the control period. Artificial digestion of blood in our experiments does not increase the amount of absorbed and utilized hemoglobin by mouth and does not raise the percentage of new hemoglobin produced per 100 grams of hemoglobin fed.

Blood sausage contains variable amounts of material other than whole blood—for example, hog fat and meat scraps and a little liver. The blood sausage used in these experiments was obtained from the Rochester Packing Company and was known to contain 22 per cent blood and 6 per cent liver. It is obvious that the liver will add something to the hemoglobin production even in these small amounts but the ingredients other than blood are relatively inert.

If we calculate the possible new hemoglobin produced on an intake of 300 grams blood sausage daily, it will be about as follows: 300 grams blood sausage is equivalent to 66 grams whole blood; 66 grams $\times$ 0.18 = 11.9 grams hemoglobin ingested or 1.2 grams hemoglobin utilized or 8.4 grams output per week. The average output per week we may say is between 20 and 25 grams hemoglobin per week above control periods. We can explain about 40 per cent of the new formed hemoglobin as due to the blood contained in the sausage. The remaining 60 per cent must be due to the liver and meat scraps included in this commercial product.

The output of new hemoglobin per week above control levels in the first experiment (table 182, dog 25–46) is about 14 grams on an intake of 200 grams blood sausage daily. The next experiment (dog 24–49) shows an output of 25 grams hemoglobin per week on an intake of 300 grams blood sausage daily. The last experiment (dog 24–45) shows an output of about 39 grams hemoglobin per week on an intake of 400 grams blood sausage per week. This last dog has an abnormally high output of hemoglobin on all diets. There is no noteworthy change in the color or hemoglobin indices.

Table 183 shows the influence of liberal feeding of cooked veal—250 to 400 grams daily. In the first experiment (dog 24–25) the hemoglobin output amounts to about 17 grams per week on an intake of 250 grams veal per day. The second experiment (dog 24–26) shows an hemoglobin output of about 12 grams hemoglobin per week on an intake of 300 grams veal daily. The last experiment (dog 24–22) shows a hemoglobin output of about 15 grams hemoglobin above control periods on a large intake of 400 grams veal daily.

These results are somewhat more uniform than those previously reported for beef muscle (3) and the output level is somewhat more favorable in response to the veal diet. The beef is much richer in muscle hemoglobin than the veal so that this substance would seem to be of minor importance in muscle feeding. The young growing muscle (veal) may have in storage

TABLE 183

Veal or calf skeletal muscle

DIET PERIODS 1 WEEK EACH	FOOD CONS.	WT.	PLASMA VOL.	RBC	COLOR INDEX	HB. INDEX	RBC HEMAT.	BLOOD HB. LEVEL	HB. REMOVED BLED
Food, grams per day	*per cent*	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*grams*
Dog 24–25 Bull, male, adult									
Bread 425, salm. 50	100	14.1	878	4.7	0.44	1.92	21.4	41	1.1
Veal 250, bread 250*	100	14.3	854	4.9	0.54	1.96	24.6	48	12.6
Veal 250, bread 250*	100	14.1	815	5.1	0.50	2.13	24.0	51	1.4
Bread 450, salm. 50	100	13.8	812	6.1	0.49	2.17	20.7	45	20.7
Bread 450, salm. 50	100	13.1	768	4.8	0.49	1.94	24.3	47	1.4
Dog 24–26 Bull, male, adult									
Bread 300, salm. 75	90	10.3	612	5.1	0.44	1.97	22.9	45	1.4
Veal 300, bread 200*	91	10.5	618	5.7	0.44	1.97	24.8	49	12.8
Veal 300, bread 200*	100	10.8	598	5.1	0.51	1.91	23.2	44	12.4
Bread 300, salm. 75	94	10.5	598	4.9	0.45	1.94	22.8	44	1.3
Bread 300, salm. 75	100	10.4	640	4.5	0.53	2.00	23.9	48	1.4
Dog 24–22 Coach, female, adult									
Bread 300, salm. 50	100	11.3	706	4.7	0.43	1.80	22.1	40	1.1
Veal 400	100	11.0	664	5.6	0.44	1.83	26.7	49	1.3
Veal 400	100	10.3	708	6.6	0.42	1.82	23.9	43	14.5
Bread 350, salm. 50	100	10.8	676	5.2	0.46	1.84	25.9	48	1.3
Bread 350, salm. 50	100	10.8	742	5.0	0.54	2.03	23.1	47	13.3

* Veal broth added to mixture.

more building stones suitable for new hemoglobin construction. We may compare also the reaction to pig muscle feeding (6) which averages fairly close to the output figures of table 183 but falls a little below. Veal stands on a par with beef or pig heart in its potency for new hemoglobin production.

Table 184 shows the influence of egg yolk, egg white and whole egg upon the hemoglobin regeneration. The effect is minimal in spite of a large intake and there is no conspicuous difference between the egg yolk

TABLE 184

Egg yolk and egg white

DIET PERIODS 1 WEEK EACH	FOOD CONS.	WT.	PLASMA VOL.	RBC	COLOR INDEX	HB. INDEX	RBC HEMAT.	BLOOD HB. LEVEL	HB. REMOVED BLED
Food, grams per day	*per cent*	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*grams*
Dog 18–126 Bull, male, adult									
Butter 50, bread 350	47	13.3	646	3.5	0.68	2.42	19.6	47	0
Egg alb. 150, br. 350	43	13.0	634	3.4	0.72	2.54	19.2	49	0
Egg alb. 150, br. 350	68	13.3	646	3.6	0.87	2.36	16.7	39	14.6
Bread 400	58	12.8	638	3.2	0.58	2.21	16.8	37	0
Bread 400	66	12.6	644	4.1	0.60	2.46	20.1	49	0
Yolk 85, bread 350	96	13.3	610	2.9	0.93	2.59	16.2	42	11.0
Yolk 85, bread 350	67	13.2	622	3.8	0.52	2.44	16.2	40	0
Bread 400	45	13.3	596	3.9	0.53	2.34	17.8	42	0
Dog 19–95 Bull, male, adult									
Egg alb. 150, br. 400	92	15.3	968	2.6	0.60	2.19	14.2	31	0
Egg alb. 150, br. 400	87	15.4	890	3.6	0.57	2.46	16.8	41	0
Yolk 85, bread 400	83	15.5	850	4.3	0.61	2.42	19.1	46	9.3
Yolk 85, bread 400	83	15.7	921	3.0	0.60	2.19	16.4	36	0
Yolk 85, bread 400	77	15.3	827	4.8	0.47	2.14	20.9	45	0
Bread 450	71	15.0	811	4.0	0.62	2.50	19.7	49	0
Butter 50, bread 400	73	15.1	824	4.9	0.48	2.40	19.6	47	0
Butter 50, bread 400	85	14.7	806	4.8	0.46	2.26	19.4	44	0
Eggs 200, bread 350	97					2.70	21.8	59	0
Eggs 200, bread 350	88	15.0	842	3.9	0.49	2.34	16.2	38	13.3
Bread 450	78	14.7	878	3.8	0.54	2.07	19.8	41	0

and egg white. For feeding the eggs were hard boiled and the yolks and whites separated. The material was then ground up and mixed as usual with the standard bread.

These experiments were done several years ago and are not as satis-

factory in some respects as more recent ones. The control periods are not adequate and the food intake not as carefully calculated as in more recent experiments. The dogs' weights are constant yet more or less food is left unconsumed which of course means too many calories in the form of bread. The hemoglobin removed during blood volume and hemoglobin determinations is not listed in the tables. From other tables it is readily seen that this amounts to 1 to 2 grams each week.

TABLE 185

Chicken skeletal muscle

DIET PERIODS 1 WEEK EACH	FOOD CONS.	WT.	PLASMA VOL.	RBC	COLOR INDEX	HB. INDEX	RBC HEMAT.	BLOOD HB. LEVEL	HB. REMOVED BLED
Food, grams per day	*per cent*	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*grams*
Dog 24–49 Bull, female, adult									
Bread 500, salm. 75.....	94	16.1	947	5.1	0.42	2.00	21.7	43	**1.3**
Bread 400, salm. 100.....	99	16.0	902	6.3	0.35	1.91	23.2	44	**2.9**
White meat 250, br. 350....	98	16.6	985	6.0	0.44	2.10	21.5	45	**15.8**
White meat 250, br. 350....	100	16.4	1006	6.8	0.34	1.97	23.6	47	**1.4**
Bread 400, salm. 75.....	84	16.1	826			1.79	24.3	43	**16.4**
Bread 375, salm. 100.....	87	15.7	952	5.8	0.35	1.73	23.1	40	**1.1**
Dog 24–25 Bull, male, adult									
Bread 450, salm. 75.....	100	13.3	828	5.0	0.43	1.88	22.8	43	**2.6**
Dark meat 250, br. 300.....	100	13.8	812	5.2	0.46	1.90	25.5	48	**1.4**
Dark meat 250, br. 300.....	100	14.0	836	6.6	0.39	2.00	26.0	52	**16.1**
Bread 450, salm. 75.....	100	14.4	766			1.85	18.8	35	**12.6**
Bread 450, salm. 75.....	90	13.9	815	4.8	0.44	1.88	22.4	42	**2.2**

$$\text{Hemoglobin index} = \frac{\text{Hemoglobin per cent}}{\text{Red cell hematocrit per cent}}.$$

In spite of these technical defects it is clear that eggs do not contain any large amount of substances favorable for new hemoglobin production. We calculate the new formed hemoglobin in these experiments about as follows: The first experiment (dog 18–126, table 184) shows a production of about 7 grams hemoglobin per week on egg white and but 2 grams hemoglobin per week on egg yolk. The daily intake of egg white and yolk is given as grams and represents the material from 6 eggs. The second experiment (dog 19–95, table 184) shows an output of about 5 grams hemoglobin per week on egg white and about 6 grams hemoglobin per

week on egg yolk. Whole egg feeding gives a hemoglobin value of about 5 grams hemoglobin per week. These figures then average close to 5 grams new hemoglobin produced per week on a sizable daily intake of whole eggs, egg yolk or egg white.

We had high hopes that egg yolk feeding might cause some constant change in the hemoglobin index and we note a definite tendency for this

TABLE 186

Chicken bones and skin

DIET PERIODS 1 WEEK EACH	FOOD CONS.	WT.	PLASMA VOL.	RBC	COLOR INDEX	HB. INDEX	RBC HEMAT.	BLOOD HB. LEVEL	HB. REMOVED BLED
Food, grams per day	*per cent*	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*grams*
Dog 24–22 Coach, female, adult									
Bread 400, salm. 75	100	12.0	738	5.1	0.39	1.96	20.5	40	**2.4**
Ch. b. & m. 250, br. 250	100	12.0	771	4.6	0.40	1.95	19.1	37	**1.0**
Ch. b. & m. 250, br. 300	100	12.0	696	5.8	0.44	1.99	21.1	42	**13.1**
Bread 400, salm. 75	90	11.8	646			2.13	18.6	40	**1.2**
Dog 21–67 Bull, male, adult									
Bread 350, salm. 100	88	11.4	671	4.2	0.51	1.96	21.8	43	**1.6**
Ch. b. & m. 250, br. 250	100	11.8	672	5.0	0.46	1.99	23.2	46	**1.6**
Ch. b. & m. 250, br. 250	92	11.9	554	5.2	0.58	1.94	15.8	31	**18.4**
Bread 350, salm. 100	83	11.9	644	3.8	0.55	1.96	21.4	42	**1.1**
Bread 350, salm. 100	79	12.1	643	4.7	0.51	2.00	24.0	48	**1.4**
Dog 24–46 Bull, female, adult									
Ch. skin 250, br. 400	100	19.2	995	5.7	0.46	1.99	22.3	44	**16.0**
Ch. skin 250, br. 400	90	19.5	1084	4.4	0.52	2.03	22.6	46	**1.2**
Bread 550, salm. 100	63	18.7	1097	5.0	0.43	1.85	23.4	43	**1.3**
Bread 450, salm. 100	95	18.9	1021	5.1	0.46	1.86	25.2	47	**1.2**

Ch. b. & m. = chicken bones and meat.

figure to fall during and just after egg yolk or whole egg feeding. But the fluctuations in the hemoglobin index are unusually great in this series of experiments and one does not feel secure in drawing any conclusions. One might suspect that egg yolk feeding might contribute especially to red cell stroma production and possibly cause an over-production of red cell matrix with fall of the hemoglobin index.

Eggs fall into the class of dairy products which contain minimal amounts of substances favorable for new hemoglobin production in anemia.

After we reported (6) the surprising effect of chicken gizzard which has a potency almost equal to whole liver in promoting new hemoglobin

TABLE 187

Gelatin feeding

DIET PERIODS 1 WEEK EACH	FOOD CONS.	WT.	PLASMA VOL.	RBC	COLOR INDEX	HB. INDEX	RBC HEMAT.	BLOOD HB. LEVEL	HB. REMOVED BLED
Food, grams per day	*per cent*	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*grams*
Dog 24–45 Bull, female, adult									
Bread 500	100	17.7	1104	5.1	0.45	2.00	22.9	46	**1.3**
Gelatine 50, br. 450	100	18.4	1051	5.6	0.46	2.08	24.0	50	**13.3**
Gelatine 50, br. 450	100	18.2	1008	5.2	0.51	2.08	20.7	64	**13.5**
Bread 450	100	17.8	937	6.1	0.42	2.13	26.2	56	**14.1**
Bread 450	100	17.6	1067	4.9	0.51	2.05	21.5	44	**13.6**
Bread 450	100	17.0	1080	5.5	0.44	2.10	23.0	48	**1.4**
Dog 25–24 Bull, male, young adult									
Bread 400, salm. 75	100	10.9	705	4.9	0.44	1.78	24.5	43	**1.3**
Bread 400, salm. 75	100	10.8	716	4.7	0.49	1.88	24.5	46	**1.3**
Gelatine 50, br. 350	100	10.9	700	5.4	0.64	1.86	22.4	42	**14.6**
Gelatine 50, br. 350	100	10.5	667	4.7	0.51	2.08	23.1	48	**1.4**
Bread 400, salm. 50	100	10.7	702	5.1	0.41	1.84	22.9	42	**1.3**
Bread 400, salm. 50	100	10.7	710	5.0	0.43	1.90	22.7	43	**1.3**
Dog 24–49 Bull, female, adult									
Bread 450	97	15.9	935	6.0	0.37	1.78	25.2	45	**1.3**
Bread 450	100	15.9	949	5.4	0.38	1.71	25.0	41	**1.2**
Gelatine 75, br. 400	96	16.6	991	5.0	0.45	1.78	25.4	45	**1.4**
Gelatine 75, br. 400	92	16.0	831	6.6	0.45	1.90	20.9	40	**17.5**
Bread 450	96	16.5	997	5.2	0.38	1.85	21.3	39	**1.2**
Bread 450	100	16.1	948	5.4	0.45	1.87	25.9	49	**1.4**

production, we had frequent inquiries as to chicken skeletal muscle. The experiments in tables 185 and 186 give evidence that the striated muscles and bones of chickens contain no unusual factors favorable for new hemoglobin production. Adult birds were obtained from the market. They were cut up and boiled until the meat was easy to dissect. The

breast and wing meat was separated and fed as *white meat.* The leg muscles and large back muscles were separated and fed as *dark meat.* The remaining meat on ribs, back and neck was combined with the skeleton and fed (table 186). The skin and subcutaneous fat was separated and fed as *skin* (table 186).

Table 185 shows that both white and dark meat of the chicken contain equivalent amounts of substances favorable for the fabrication of new hemoglobin in this type of experimental anemia. The output per week amounts to about 12 grams hemoglobin on a daily intake of 250 grams muscle whether white or dark. This corresponds closely to the output on pig's skeletal muscle. We may recall that the breast muscle of the chicken contains no demonstrable muscle hemoglobin while by contrast the gizzard shows very high concentrations, about 700 mgm. per 100 grams muscle, and the leg muscles considerably less—about 320 mgm. per 100 grams muscle (2). It is evident that in this hemoglobin reaction to striped muscle feeding the muscle hemoglobin content plays no significant rôle.

Table 186 shows that the mixture of chicken bones and adherent meat scraps is a little less potent than the skeletal muscle alone. If we assign some of the observed reaction to the meat scraps adherent to neck, back and thorax, we have very little indeed to designate as *bone marrow effect.* The first experiment (dog 24–22, table 186) shows a weekly output of about 5 grams hemoglobin on a liberal intake of chicken bones and meat scraps. The second experiment (dog 21–67, table 186) shows about 10 grams hemoglobin output per week. The last experiment with feeding of skin and subcutaneous fat shows a weekly output of about 8 grams hemoglobin over control levels.

Table 187 shows that gelatin reacts much like beef skeletal muscle when added in large amounts to the standard bread. Its deficiency of tyrosine and tryptophane does not cause it to fail wholly in hemoglobin production in anemia. The first experiment (dog 24–45, table 187) shows an unusually active hemoglobin production of about 23 grams per week but we may explain this as due to the personal idiosyncrasy of this animal—consult tables 182 and 181. The second and third experiments in table 187 show 5 grams and 12 grams hemoglobin production respectively per week on varying amounts of gelatin by mouth. There are no noteworthy fluctuations in the color or hemoglobin indices. The plasma volume figures show constant levels which is important as in some experiments with gelatin we may observe gastro-intestinal disturbances and shrinkage of plasma volume which leads to a rise in the hemoglobin level without any actual new hemoglobin production. This may lead to mistakes in interpretation if the plasma volume figures are not available.

CONCLUSIONS

Liver sausage as tested in these experiments shows a moderately high potency for new hemoglobin production which depends upon the amount of liver contained in the sausage. The output of new hemoglobin averages about 40 to 50 grams per 2-week period.

Blood sausage also is quite potent in these experimental anemias in dogs. It may run as much as one-half the potency of whole liver. Its potent factors are whole blood, meat scraps and a little liver. It is probable that the contained blood is responsible for almost half the total effect.

Liver and blood sausage deserve careful study as to their applicability in various human anemias. As accessory diet factors they may prove to be quite valuable.

Calf skeletal muscle (veal) is as potent as any skeletal muscle so far tested and is in the class with beef heart. In these standard dogs the production of hemoglobin will average close to 25 grams per 2-week period which is about one-fourth the average values for liver.

Egg yolk and egg white are relatively inert and the average hemoglobin production is about 10 grams per 2 weeks over control levels.

Chicken skeletal muscle (white or dark) is a little less potent than calf muscle; chicken bones and skin still a little below chicken muscle.

Gelatin feeding in large amounts will increase somewhat the hemoglobin output above control levels. It corresponds closely to the effect of beef muscle. We may say that gelatin adds something other than tyrosine or tryptophane to the standard bread ration which enables the body to fabricate considerable new hemoglobin.

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BLOOD REGENERATION IN SEVERE ANEMIA

XIX. Influence of Spinach, Cabbage, Onions and Orange Juice

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The ebb and flow of opinion concerning the importance of vegetables and fruits in the diet makes a confused but interesting chapter in the history of body nutrition. The same statement applies to general pigment metabolism including anemia. Some vegetables have been held in high favor as contrasted with others when considered from the viewpoint of therapy in anemia. This applies especially to *spinach* and other chlorophyll rich vegetables which have reputations better than they deserve, based on the assumption that animals can utilize chlorophyll to construct new hemoglobin. This belief is based largely on similarity of chemical structure when hemoglobin and chlorophyll are compared. Possibly herbivora can utilize chlorophyll to build up new hemoglobin but the evidence is strong that dogs (2) cannot make use of this green pigment to reconstruct new hemoglobin even under the stress of a long continued anemia. On the whole vegetables are fairly uniform in their reaction in anemic dogs and possess but a very modest amount of substances which are favorable for new hemoglobin production. The chlorophyll content seems to be in no way related to this reaction.

Fruits by contrast with vegetables present every degree of potency. Some fruits are quite inert and others contain potent materials which exceed the concentration of these materials in meat products, like beef heart and pancreas. For example, raspberries are inert while apricots and peaches (3) are highly potent in anemia, much more so than any vegetables. If we rate the hemoglobin production in standard anemia as 80 to 100 grams hemoglobin per 2 weeks for *liver* feeding we find *apricots* and peaches rate about 40 to 45 grams hemoglobin per 2 weeks and *spinach* and cabbage about 20 to 25 grams hemoglobin per 2 weeks; in a word, about a 4–2–1 ratio.

Salts play an important part in this reaction effected by fruits and vegetables. It has been shown (1) that apricot ash contains almost all the potency of the fresh fruit. Obviously this is due to a salt mixture which

is known to contain iron and copper in appreciable amounts. It is highly probable that some of the spinach and cabbage effect on hemoglobin regeneration is a salt effect. This leaves little enough to attribute to chlorophyll even if we disregard the fact that spinach differs very little in its effect in anemia from other vegetables poor in chlorophyll.

It can be stated with security that no investigator would pretend to write specifications for the metabolic reaction of any single food material, based solely upon a complete knowledge of its chemical makeup. This applies to the effect of various food materials upon hemoglobin regeneration in severe anemia. There are always surprises in store for the investigator and the explanation of unexpected reactions often must await future knowledge. Many fruits are relatively or quite inert yet apricots are more potent than any meat products except liver, kidney and gizzard. Much of this apricot potency is a salt effect and demonstrable in the apricot ash. The common grains are inert but some may be discovered which are potent. The vegetables so far tested are rather uniform and but slightly potent. Dairy products and eggs are surprisingly lacking in potent factors.

The riddle of the distribution of these potent factors in the various body tissues and organs remains to be solved. It would appear that bone marrow and spleen ought to contain more materials suitable for red cell and hemoglobin construction than any body tissue other than the liver which is so intimately related to all pigment metabolism. Yet spleen and bone marrow fall far below kidney and gizzard when compared in standard anemic dogs and a considerable portion of the spleen-marrow reaction may well be due to the hemoglobin included in the material as fed.

Vitamines would seem to be excluded from these particular reactions on a number of counts. In the first place there is adequate and high vitamine intake on the standard bread ration which contains abundant yeast, cod liver oil and tomatoes. Excess intake of eggs, milk and butter has very little effect upon hemoglobin regeneration. Wheat germ has been tested with negative result and orange juice tabulated below is almost inert.

Vegetable pigments are always intriguing but in these experiments to date have no influence on hemoglobin regeneration. This applies to the pigments in beets, carrots and red cabbage (table 193). The same may be said for the various pigments in fruits—for example grapes, apricots, berries and prunes.

EXPERIMENTAL OBSERVATIONS. All methods were described in paper XVI of this series. The calculated values for hemoglobin production were estimated as described under methods.

Table 191 gives an excellent comparison between spinach feeding and spinach plus iron. This is to be compared with the experiments in paper XVI above to show the average hemoglobin output on this amount of iron by mouth.

Spinach has an average effect on hemoglobin regeneration in this experiment (table 191). We estimate this output as 13 grams hemoglobin per week and the contrast on spinach plus iron is notable. We find a total hemoglobin output of 45 grams per week over control levels. The difference or 32 grams hemoglobin we may properly attribute to the iron therapy. The average hemoglobin output per week on an optimum intake of iron salt is about 25 grams hemoglobin per week. This type of

TABLE 191

Spinach alone and fed with iron

DIET PERIODS 1 WEEK EACH	FOOD CONS.	WT.	PLASMA VOL.	RBC	COLOR INDEX	HB. INDEX	RBC HEMAT.	BLOOD HB. LEVEL	HB. REMOVED BLED
Food, grams per day	*per cent*	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*grams*
Dog 23–3 Bull, female, adult									
Bread 250*	100	13.2	734	3.6	0.58	2.22	19.1	42	**1.3**
Spinach 225, bread 250*	100	13.1	780	4.5	0.52	2.22	21.2	47	**1.4**
Spinach 300, bread 250*	100	13.0	672	5.6	0.49	2.42	23.4	57	**13.6**
Bread 300*	100	13.3	760	5.2	0.48	2.29	21.8	50	**13.5**
Bread 300*	100	13.4	722	4.3	0.52	2.31	19.3	45	**1.3**
Spinach 300, Fe 40**	100	13.2	702	5.1	0.57	2.46	26.9	66	**28.0**
Spinach 300, Fe 40**	100	13.1	690	5.1	0.68	2.46	24.5	60	**29.9**
Bread 350*	100	13.4	694	3.8	0.71	2.46	21.3	52	**16.3**
Bread 350*	100	13.6	726	4.4	0.66	2.42	22.1	53	**16.2**
Bread 350*	100	13.7	728	3.6	0.71	2.48	17.5	43	**10.6**
Bread 350*	100	14.1	780	3.6	0.64	2.54	18.1	46	**1.5**

* Salmon 100 grams and Klim 25 grams added to daily diet.

** Bread 250 grams and salmon 100 grams added to daily diet.

Iron given in the form of ferric chloride tabulated as milligrams of metal.

$$\text{Hemoglobin index} = \frac{\text{Hemoglobin per cent}}{\text{Red cell hematocrit per cent}}.$$

experiment gives evidence that the iron contained in the spinach is of relatively little importance as it would be submerged by these large doses of iron salts. The hemoglobin index averages somewhat higher during and after the iron therapy as contrasted with the fore period.

Table 192 shows three typical experiments with boiled cabbage which was ground up and mixed thoroughly with the standard bread. In spite of this large intake of a food rather unusual for the dog we note no clinical disturbance, no diarrhea and no changes in blood volume beyond

the usual normal fluctuations. The figures for new hemoglobin output per week are quite uniform and amount to 13 grams, 15 grams and 13 grams hemoglobin respectively in the three experiments given in table 192. The intake of cabbage varies from 100 to 250 grams daily which is the maxi-

TABLE 192

White cabbage

DIET PERIODS 1 WEEK EACH	FOOD CONS.	WT.	PLASMA VOL.	RBC	COLOR INDEX	HB. INDEX	RBC HEMAT.	BLOOD HB. LEVEL	HB. REMOVED BLED
Food, grams per day	*per cent*	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*grams*
Dog 25–23 Bull, male, adult									
Bread 250, salm. 100.....	100	14.1	806	4.7	0.48	1.91	23.4	45	1.3
Wh. cbg. 100, br. 225*.....	98	13.7	794	5.6	0.39	1.79	24.5	44	1.2
Wh. cbg. 100, br. 225*.....	98	13.7	794	6.6	0.47	1.93	24.6	48	15.5
Bread 275, salm. 100, Klim 25	76	13.0	785	5.7	0.45	1.82	26.8	49	12.8
Bread 235, Klim 25‡.....	97	13.6	788	5.3	0.44	1.87	25.2	47	1.4
Dog 25–24 Bull, male, young adult									
Bread 500.....	100	11.2	700	4.2	0.43	1.90	19.0	36	1.0
Wh. cbg. 200, br. 425†.....	76	10.8	696	5.3	0.45	1.90	25.2	48	1.3
Wh. cbg. 100, br. 425, Kl. 25†	100	11.2	650	5.4	0.45	1.97	24.9	49	1.6
Bread 500, salm. 50.....	100	11.1	644	6.7	0.42	2.10	24.0	50	13.6
Bread 500, salm. 75.....	100	11.2	690	6.0	0.43	1.84	25.5	47	10.4
Bread 500, salm. 75.....	100	11.3	738	5.2	0.45	2.08	22.6	47	1.4
Dog 24–49 Bull, female, adult									
Bread 450, salm. 75.....	90	16.3	946	5.6	0.44	1.96	25.0	49	1.5
Wh. cabbage 250, br. 400..	98	16.2	897	5.3	0.48	2.00	26.5	53	14.9
Wh. cabbage 250, br. 450..	96	16.0	876	6.4	0.50	1.94	26.1	51	21.1
Bread 500, salm. 75.....	94	16.1	947	5.1	0.42	2.00	21.7	43	1.3
Bread 400, salm. 100.....	99	16.0	902	6.3	0.35	1.91	23.2	44	2.9

* Salmon 100 grams added to daily diet.

† Salmon 75 grams added to daily diet.

‡ Salmon 50 grams and meat scraps 50 grams added to daily diet.

mum intake for our animals. An intake of 200 grams boiled cabbage represents about 275 grams fresh cabbage. This hemoglobin output on cabbage is identical with that on spinach—compare table 191 and many experiments in paper IV (2) of this series.

Table 193 shows similar results due to different amounts of boiled red cabbage. The food intake is satisfactory in spite of the unusual ration and large amount of this vegetable. The hemoglobin output amounts to

TABLE 193

Red cabbage

DIET PERIODS 1 WEEK EACH	FOOD CONS.	WT.	PLASMA VOL.	RBC	COLOR INDEX	HB. INDEX	RBC HEMAT.	BLOOD HB. LEVEL	HB-REMOVED BLED
Food, grams per day	*per cent*	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*grams*
Dog 21–67 Bull, male, adult									
Bread 300, salm. 75.....	100	11.2	614	4.7	0.52	1.85	26.2	49	**1.5**
Bread 350, salm. 75.....	80	10.9	598	5.0	0.46	1.98	23.5	46	**1.6**
Red cabbage 100, br. 300*..	92	11.2	611	5.4	0.54	2.00	22.1	44	**16.1**
Red cabbage 100, br. 300**.	100	11.4	633	5.1	0.49	1.95	25.8	50	**1.7**
Bread 350, salm. 100.....	86	11.4	598	5.3	0.52	1.94	20.8	40	**14.0**
Bread 350, salm. 100.....	88	11.4	671	4.2	0.51	1.96	21.8	43	**1.6**
Dog 24–59 Bull, male, young adult									
Br. 450, salm. 50, Klim 50...	100	13.9	842	5.8	0.42	1.84	26.4	49	**1.3**
Red cabbage 200, br. 400**.	100	14.0	848	6.2	0.41	1.89	26.9	51	**1.5**
Red cabbage 200, br. 400**.	100	14.3	904	7.7	0.37	1.85	27.2	50	**13.1**
Bread 500, salm. 75.....	100	14.4	897	7.0	0.40	1.94	26.2	51	**12.6**
Bread 550, salm. 75.....	100	14.4	900	5.6	0.45	1.85	20.2	37	**12.3**
Bread 550, salm. 75.....	100	14.5	983	5.9	0.35	1.95	21.0	41	**1.0**
Dog 24–22 Coach, female, adult									
Bread 375, salm. 75.....	100	11.5	676	5.1	0.41	1.93	21.7	42	**1.3**
Red cabbage 200, br. 325**.	100	11.4	661	5.5	0.45	2.00	25.0	50	**1.4**
Red cabbage 200, br. 375**.	100	11.8	750	5.2	0.55	1.92	28.0	54	**15.3**
Bread 400, salm. 75.....	100	11.8	723	6.3	0.45	2.10	22.9	48	**13.9**
Bread 400, salm. 75.....	100	12.0	738	5.1	0.39	1.96	20.5	40	**2.4**

* Salmon 75 grams and Klim 15 grams added to daily diet.

** Salmon 75 grams added to daily diet.

14 grams, 11 grams and 11 grams per week respectively in the 3 experiments given in table 193. This pigment in red cabbage like the pigments in many other vegetables and fruits is inert in these experiments.

Table 194 shows three satisfactory experiments with boiled onions.

This vegetable comes very close to zero potency as measured by new hemoglobin production in anemia. Whatever good or bad may be said about the onion its strength is not measured in terms of grams of hemoglobin produced in anemia. The first experiment (dog 24–42, table 194) shows an output of 8 grams hemoglobin per week. The last two experiments in table 194 show but 2 grams hemoglobin production per week.

TABLE 194

Onions

DIET PERIODS 1 WEEK EACH	FOOD CONS.	CT.	PLASMA VOL.	RBC	COLOR INDEX	HB. INDEX	RBC HEMAT.	BLOOD HB. LEVEL	HB. REMOVED BLED
Food, grams per day	*per cent*	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*grams*
Dog 24–42 Bull, male, adult									
Bread 600	100	16.3	866	6.1	0.41	1.77	28.4	50	0
Onions 200, bread 450	100	15.8	852	6.0	0.43	1.80	29.0	52	11.6
Onions 200, bread 450	100	15.7	792	6.1	0.45	1.72	26.2	45	12.5
Bread 600	100	16.0	899	5.2	0.45	1.82	25.7	47	1.1
Dog 21–23 Bull, female, adult									
On. 200, br. 450, salm. 75	56	16.5	1028	6.1	0.40	1.89	26.2	49	1.6
On. 150, br. 450, salm. 100	88	17.2	880	5.9	0.41	1.79	26.8	48	1.6
On. 100, br. 400, salm. 100	97	16.8	908	6.3	0.41	2.13	22.5	48	13.5
Bread 550, salm. 50	59	18.0	1180	5.2	0.40	1.82	22.6	41	1.2
Dog 24–26 Bull, male, young adult									
Bread 450, salm. 50	63	10.9	598	5.6	0.48	1.93	27.7	54	0
Bread 450, salm. 50	78	11.3	655	5.5	0.54	2.00	26.2	52	9.6
Onions 200, bread 400	76	11.1	663	4.5	0.57	1.95	26.4	51	0
Onions 200, bread 350	91	10.9	642	4.9	0.57	1.85	24.3	45	11.7
Bread 450, salm. 50	82	10.8	652	4.6	0.45	1.79	23.1	41	0
Bread 450, salm. 50	63	10.1	622	4.9	0.46	1.78	25.3	45	0

One experiment was carried through 3 weeks and the last one for 2 weeks as usual. This output comes within the physiological fluctuations observed in these experiments during long bread diet periods. We may say that these last two experiments in table 194 are practically negative. As a matter of fact at one time we suspected that onions actually inhibited hemoblogin production and they may actually contain some factor which does inhibit new hemoglobin production. Some of our experiments sug-

gest this possibility but the experimental data are insufficient. We may note a slight tendency for the hemoglobin index to fall during and after periods of abundant vegetable feeding.

Table 195 shows the slight influence of fresh orange juice upon new hemoglobin production. Fresh undiluted orange juice, 60 cc. daily, was given for a period of 2 weeks. The first experiment (dog 24–22, table 195) shows a hemoglobin production uninfluenced by the orange juice and ex-

TABLE 195

Orange juice

DIET PERIODS 1 WEEK EACH	FOOD CONS.	WT.	PLASMA VOL.	RBC	COLOR INDEX	HB. INDEX	RBC HEMAT.	BLOOD HB. LEVEL	HB. REMOVED BLED
Food, grams per day	*per cent*	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*grams*
Dog 24–22 Coach, female, adult									
Bread 300*	100	12.8	822	4.9	0.43	2.06	20.4	42	1.1
Bread 300*	100	12.8	813	4.7	0.48	2.01	22.4	45	1.4
Orange juice 60, br. 300*	90	13.1	828	4.1	0.40	1.97	16.5	33	1.0
Orange juice 60, br. 300*	95	12.9	745	4.6	0.39	1.91	18.6	36	1.1
Bread 300*	86	12.8	732	4.6	0.47	2.08	20.6	43	1.2
Bread 300*	92	12.6	728	5.2	0.42	2.05	21.4	44	1.3
Bread 300*	94	12.6	796	5.5	0.36	2.02	19.4	39	1.2
Dog 24–25 Bull, male, adult									
Bread 350*	64	15.1	792	3.3	0.52	2.16	15.8	34	1.6
Orange juice 60, br. 200*	97	14.8	840	4.2	0.51	2.27	18.7	43	1.2
Orange juice 60, br. 200*	93	14.5	741	3.9	0.51	2.16	18.3	40	1.2
Bread 250*	100	14.5	866	4.7	0.47	2.16	19.6	42	12.4

* Salmon 100 grams and Klim 25 grams added to daily diet.

$$\text{Hemoglobin index} = \frac{\text{Hemoglobin per cent}}{\text{Red cell hematocrit per cent}}.$$

actly like long control periods of standard bread diet. The second experiment (dog 24–25, table 195) shows a slight increase in hemoglobin—about 9 grams per week above control levels.

CONCLUSIONS

Spinach and cabbage (red and white) show but a moderate effect on hemoglobin regeneration in standard anemia experiments. We may say that 10 to 12 grams hemoglobin per week above control levels represent their influence on blood regeneration.

Iron in optimum dosage added to the spinach ration may give complete summation—that is, the total effect as a rule will amount to the moderate spinach effect plus the larger iron salt effect. This may indicate that the spinach effect is not due to iron in this vegetable.

Onions are almost inert when tested in these anemia experiments.

Orange juice likewise is almost inert under these experimental conditions.

There is no evidence that various pigments which may be abundant in many fruits and vegetables have any influence on hemoglobin regeneration.

Chlorophyll likewise appears to be wholly inert in these experiments with long continued anemia in dogs.

It seems extremely unlikely that vitamines are in any way concerned with new hemoglobin production under these conditions.

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BLOOD REGENERATION IN SEVERE ANEMIA

XX. Conservation of Sheep and Goose Hemoglobin Given Intravenously to Form Dog Hemoglobin

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These experiments give convincing evidence that the anemic dog is able to conserve for new hemoglobin construction about all of the injected foreign hemoglobin. Hemoglobins from sheep and geese are alike in this respect and we must assume they are broken down to intermediates and recast within the body to form new dog hemoglobin. The intravenous intake in all experiments is 25 to 30 grams of sheep or goose hemoglobin and the new hemoglobin production in these standard dogs runs from 29 to 46 grams hemoglobin during the injection and after periods. These large amounts give sufficient security that the injected hemoglobin is used in some way to effect this large increase in the production of hemoglobin during controlled standard bread diet periods. It is interesting to speculate how and where this interesting conservation may take place. Some of these possibilities are discussed below but they lead to a consideration of the complex interactions within the body about which our knowledge is sadly inadequate.

We have published experiments (2) to show how beautifully complete and efficient is the conservation by the anemic dog of injected dog hemoglobin. This reaction is similar whether the hemoglobin is given intravenously or intraperitoneally. This may be looked upon as a demonstration of ways and means which the body possesses to insure conservation of valuable material—for example waste products from red blood cell destruction or daily wear and tear in the circulation. We were conservative in our statement that the anemic dog recovered not less than 80 to 90 per cent of the injected dog hemoglobin. Moreover a pancreatic digest of dog red blood cells given intravenously showed a conservation of about 40 per cent of the original hemoglobin digested. This indicated that the body could conserve various split products coming from hemoglobin digestion and construct new hemoglobin from these building stones. We

do not know whether all dog hemoglobin given intravenously is first broken down and then rebuilt into new hemoglobin in the routine as prescribed by body metabolism. Probably some of it is thus broken down before utilization but the evidence suggests that some dog hemoglobin may be utilized direct and incorporated into the immature red cell matrix. Evidently the usual construction of hemoglobin within the body is a synthetic grouping of amino acids and other factors coming from food intake, red cell wear and tear or tissue katabolism. Possibly all hemoglobin utilization must go through this cycle. Surely the experiments in this paper can be explained only by a katabolism of the foreign hemoglobin and subsequent construction of new dog hemoglobin.

When dog hemoglobin is given intravenously we note that the output of new hemoglobin is a little more prompt than in the case of the foreign hemoglobin. The new formed dog hemoglobin after sheep or goose hemoglobin injection may not appear completely until two or even three weeks after the completion of the injections. With dog hemoglobin injection this new hemoglobin output is usually complete within one week after all injections have been given. This may indicate that the katabolism of these foreign hemoglobins is a little more difficult and time consuming but in the end the body utilizes all of this material. It is also possible that in the case of dog hemoglobin a portion of the injected hemoglobin is utilized direct by the marrow cells and only a part broken down for the customary synthesis of new hemoglobin. This might explain the shorter interval for complete synthesis of new hemoglobin following dog hemoglobin injection. It is useless to speculate as to the site of this interesting katabolism but one suspects the reticulo-endothelial system. We have always believed that the liver epithelium plays an all important rôle in the building up of these materials into the prehemoglobin substance which is incorporated in the growing red cells of the marrow.

Experimental observations. The general methods are described in paper XVI of this series. The preparation of the foreign hemoglobin and desensitization of animals are described in a recent paper on hemoglobin renal thresholds (1). As a rule the foreign hemoglobin was given intravenously, about 2 grams a day, the total in grams being recorded each week in the tables. This daily injection is below the threshold level so that no hemoglobin is lost through the kidney.

Table 201 shows 2 similar experiments with sheep hemoglobin intravenously. The first experiment is a little less satisfactory than the second. The first dog (25–23, table 201) was a little upset by the sheep hemoglobin injections and we note a subnormal food intake necessitating the addition of meat scraps to the diet of the second week. The total injection is 31.6 grams sheep hemoglobin plus 1.6 gram for desensitization and the total output is about 46 grams dog hemoglobin. At first sight this might suggest

a stimulation of the bone marrow with some over production. However we believe that in this severe continuous anemia the hematopoietic tissues are working under maximal stimulus. The output exceeds the intake by

TABLE 201

Sheep hemoglobin given intravenously. Conservation to form dog hemoglobin

DIET PERIODS 1 WEEK EACH	FOOD CONS.	WT.	PLASMA VOL.	RBC	COLOR INDEX	HB. INDEX	RBC HEMAT.	BLOOD HB. LEVEL	HB. REMOVED BLED
Food, grams per day	*per cent*	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*grams*
Dog 25–23 bull, male, adult									
Bread 350, salm. 100*.....	100	14.3	773	5.6	0.40	1.88	23.7	44	**1.4**
Bread 350, salm. 100*.....	92	14.4	812	6.7	0.34	2.01	23.0	46	**1.4**
Sheep Hb. 17.7 gm. total +.	64	13.6	800	6.4	0.38	1.88	26.3	49	**1.5**
Sheep Hb. 13.9 gm. total**.	91	14.0	788	7.7	0.42	2.14	27.0	58	**16.3**
Bread 300, salm. 100*.....	100	13.9	802	6.6	0.46	2.05	22.4	46	**16.0**
Bread 300, salm. 100*.....	100	14.1	826	5.8	0.47	2.16	23.9	52	**11.0**
Bread 300, salm. 100*.....	100	14.4	860	5.8	0.44	2.11	21.4	45	**11.3**
Bread 300, salm. 100*.....	100	14.5	850	5.3	0.42	2.00	22.3	45	**1.4**
Dog 23–3 bull, female, adult									
Bread 225, salm. 100*.....	98	13.1	782	5.0	0.46	2.16	21.3	46	**1.3**
Sheep Hb. 8.1 total ++...	100	13.0	732	4.1	0.55	2.05	21.7	45	**1.3**
Sheep Hb. 12.1 total ++..	100	13.0	712	4.7	0.55	2.27	17.7	40	**11.6**
Sheep Hb. 11.0 total ++..	100	13.1	728	4.4	0.55	2.33	20.5	48	**1.5**
Bread 225, salm. 100*.....	100	13.2	731	5.2	0.53	2.46	20.6	51	**15.7**
Bread 250, salm. 100*.....	100	12.9	645	3.8	0.67	2.38	21.1	50	**12.4**
Bread 250, salm. 100*.....	100	12.9	695	3.7	0.57	2.31	18.3	42	**1.2**
Bread 250, salm. 100*.....	100	13.0	713	4.4	0.55	2.34	20.4	48	**1.4**

Dog 25–23 total intravenous intake 33.2 grams hemoglobin—total output 46 grams hemoglobin.

Dog 23–3 total intravenous intake 32.4 grams hemoglobin—total output 33 grams hemoglobin.

* Klim 25 grams added to daily diet.

** Bread 300, meat scraps 50, daily diet.

\+ Bread 350, salmon 100, daily diet.

++ Bread 225, salmon 100, Klim 25, daily diet.

about 13 grams hemoglobin but this excess is spread over a period of 5 weeks or a weekly excess of 2.5 grams above the average control bread diet level of 2 grams. In some animals we may observe a similar reaction during long control bread diet periods. As this is an unusual reaction we

prefer to explain this over production in this fashion rather than by some unknown stimulus coming from the foreign hemoglobin.

The second experiment (dog 23–3, table 201) shows uniform clinical conditions throughout and satisfactory food consumption. The total intake of sheep hemoglobin is 31.2 grams plus 1.2 gram for desensitization and the total output is 33 grams dog hemoglobin. The hemoglobin output is figured as described in paper XVI. We note a suggestive rise in the hemoglobin index in this experiment. There is a distinct tendency in this direction in all experiments including many with dog hemoglobin

TABLE 202

Goose hemoglobin given intravenously. Conservation to form dog hemoglobin

DIET PERIODS 1 WEEK EACH	FOOD CONS.	WT.	PLASMA VOL.	RBC	COLOR INDEX	HB. INDEX	RBC HEMAT.	BLOOD HB. LEVEL	HB. REMOVED BLED
Food, grams per day	*per cent*	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*grams*
Dog 24–2 bull, male, adult									
Bread 350, salm. 100.....	100	14.8	925	4.2	0.48	2.22	18.2	40	**1.3**
Bread 350, salm. 100.....	100	14.9	900	4.4	0.50	2.17	20.4	44	**1.4**
Bread 350, salm. 100.....	100	15.0	909	5.1	0.48	2.17	22.7	49	**1.4**
Goose Hb. 13.8 gm. total*.	100	15.2	878	5.9	0.46	2.21	22.6	50	**15.0**
Goose Hb. 13.9 gm. total*.	100	15.2	936	5.4	0.49	2.17	18.7	41	**15.6**
Bread 350, salm. 100.....	100	15.4	960	5.5	0.50	2.38	23.6	56	**16.3**
Bread 350, salm. 100.....	100	15.6	904	4.9	0.53	2.21	20.0	44	**14.7**
Bread 350, salm. 100.....	100	16.0	952	4.0	0.56	2.22	20.3	45	**1.4**
Bread 350, salm. 100.....	100	15.7	966	4.1	0.59	2.19	21.9	48	**1.4**

Dog 24–2 total intravenous intake 31.7 grams hemoglobin—total corrected output 35 grams hemoglobin.

* Bread 350 grams, salmon 100, added to daily diet.

Klim 25 added to daily diet throughout entire experiment.

$$\text{Hemoglobin index} = \frac{\text{Hemoglobin per cent}}{\text{Red cell hematocrit per cent}}.$$

injection (2). This may indicate a little more rapid production of hemoglobin as compared with red cell matrix.

Table 202 gives a completely satisfactory experiment with goose hemoglobin. In all there is an intake of 27.7 grams plus 4 grams goose hemoglobin used for desensitization. The total corrected output of dog hemoglobin is about 35 grams. This output is figured as follows: During the fore period of 3 weeks there is an increase from 40 to 49 per cent hemoglobin which we estimate as equivalent to about 9 grams hemoglobin. During the injection and after period of 6 weeks we allow twice this amount or 18

grams as a surplus above the usual control level. This is deducted from the total hemoglobin output of 53 grams. The corrected output is 35 grams which is practically equivalent to the intake. Differences of 5 to 10 grams hemoglobin in the total output observed over several weeks are within the normal fluctuations observed under the most carefully controlled conditions. We cannot explain these fluctuations at present and obviously one must not place emphasis on minor fluctuations. We believe one may conclude that the output of dog hemoglobin coincides very closely with the intake of foreign hemoglobin and indicates almost complete conservation of the foreign hemoglobin.

TABLE 203

Goose hemoglobin given intravenously. Conservation to form dog hemoglobin

DIET PERIODS 1 WEEK EACH	FOOD CONS.	WT.	PLASMA VOL.	RBC	COLOR INDEX	HB. INDEX	RBC HEMAT.	BLOOD HB. LEVEL	HB. REMOVED BLED
Food, grams per day	*per cent*	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*grams*
Dog 25–23 bull, male, adult									
Bread 300, salm. 100.....	100	13.4	862	5.3	0.44	2.11	22.1	47	**1.5**
Bread 275, salm. 150.....	80	13.2	880	5.2	0.40	2.04	20.6	42	**1.3**
Bread 275, salm. 150.....	93	13.3	868	5.2	0.41	2.04	21.3	43	**1.4**
Bread 275, salm. 150.....	100	13.9	855	6.6	0.41	2.07	20.5	42	**14.6**
Goose Hb. 8.5 total*.....	85	13.0	822	5.1	0.40	2.00	20.5	41	**1.2**
Goose Hb. 13.0 total*.....	100	13.4	755	7.2	0.38	2.07	23.4	48	**12.1**
Goose Hb. 3.6 total*.....	100	13.9	803	5.4	0.43	2.07	22.3	46	**1.4**
Bread 300, salm. 100.....	100	13.6	812	5.7	0.47	2.04	23.3	48	**14.2**
Bread 300, salm. 100.....	100	13.9	748	6.0	0.42	2.03	20.5	42	**12.1**
Bread 300, salm. 100.....	100	13.6	834	5.3	0.48	2.13	19.6	42	**13.2**
Bread 360, salm. 100.....	100	13.8	786	5.2	0.40	2.16	19.3	42	**1.4**

Dog 25-23 total intravenous intake 26.6 grams hemoglobin—total corrected output 29 grams hemoglobin.

* Bread 250, salmon 150, added to daily diet.

Klim 25 added to daily diet throughout entire experiment.

Table 203 gives a second satisfactory experiment with goose hemoglobin. The total goose hemoglobin intake is 26.6 grams including 1.5 gram for desensitization and the corrected output is about 29 grams dog hemoglobin. The fore period of 4 weeks gives an output of 13 grams and there is a loss of 5 per cent in the hemoglobin level or an excess of about 8 grams hemoglobin per 4 weeks above the usual control levels. The total output during and after hemoglobin injection is 43 grams hemoglobin, less the excess corresponding to the fore period or 14 grams giving a corrected figure of 29 grams hemoglobin output which follows closely the intake level for goose hemoglobin.

CONCLUSIONS

It seems clear that the anemic dog can conserve foreign hemoglobin given intravenously and reconstruct from this material new dog hemoglobin.

It appears obvious that the goose and sheep hemoglobin must be katabolized before this material can be utilized.

All hemoglobin including dog hemoglobin given intravenously may be broken down before it is utilized in the body.

A little more time is required for the utilization of sheep and goose hemoglobin as compared with dog hemoglobin. This may indicate that a portion of injected dog hemoglobin may be utilized direct by the marrow cells without the usual cycle of disintegration and synthesis.

These experiments illustrate beautifully the careful conservation by the body of materials suitable for hemoglobin construction, given the stress and depletion of a long continued anemia due to loss of blood.

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POSSIBLE SOURCES OF ERROR IN WEIGHTS OF SMALL MUSCLES FROZEN IN LIQUID AIR

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The importance of using ice-cold killing agents in studying the chemistry of muscle has been recognized since 1907 when Fletcher and Hopkins performed their well-known experiments on the lactic acid content of frog muscle. The true values of many of the chemical constituents of muscle can only be obtained if the muscle is thoroughly chilled before grinding and adding cold reagents. Recently liquid air has come into favor for such purposes. This cooling agent is of especial value for small muscles. Eggleton (1929) in a footnote to his article on the chemical mechanism of muscular contraction remarks of liquid air "This technique is very convenient, but somewhat risky if the muscle is not small (say, less than 200 mgm.) and thin."

In a series of experiments involving the determination of total nitrogen and non-protein nitrogen of portions of frozen frog muscle of about this size, we found that the smaller samples apparently contained a higher percentage of nitrogen than the larger samples. It was evident that all samples from a given muscle should have been uniform in composition since they were taken at random from muscle which had been thoroughly ground to powder while still in the frozen state. It seemed probable, therefore, that the weights of the muscle samples used in calculating the percentage composition were at fault. The greatest possible care, however, had been exercised in weighing to the decimal of a milligram, and furthermore the discrepancy between the large and small samples was quite constant. It was felt, therefore, that mere errors in weighing could not account for the results. Because of the heavy frosting on the outside of the iron mortar holding the liquid air, the hypothesis was advanced that moisture from the air might have condensed on the larger samples in proportionately greater quantities than on the smaller ones, thus making it appear that the chemical constituents were more concentrated in the smaller samples. A series of experiments was undertaken to test this hypothesis.

In order to estimate the water content of samples of muscle, the wet

weight was found, then the sample in its weighing bottle was placed first in an oven at 80° to 100°C. for twelve hours with the lid off, and then in a vacuum desiccator for a week until the weight became constant. When the bottles were exposed to room air, care was taken to keep the lids tightly closed, since the dried material tended to take up water if exposed for any length of time. In the tables both the wet and dry weights are given, and for purposes of comparison the dry weight is also expressed in per cent.

The wet and dry weights of different sized pieces from the same muscle untreated with liquid air were first found, and also the wet and dry weights of pieces from similar muscles on opposite sides of the body (table 1). The muscle samples were in every case taken from the belly of the muscle, usually the gastrocnemius, and because of the large size of the frogs (500

TABLE 1

Unfrozen pieces of frog muscle

	WET WEIGHT	DRY WEIGHT	PER CENT DRY WEIGHT
Frog 2:			
Gastrocnemius	1.5252	0.3055	20.0
Gastrocnemius	0.6782	0.1365	20.0
Frog 3:			
Right gastrocnemius	0.1774	0.0345	19.5
Right gastrocnemius	0.3600	0.0709	19.7
Left gastrocnemius	0.8990	0.1808	19.9
Left gastrocnemius	0.7702	0.1511	20.0
Frog 1:			
Left gastrocnemius	1.3090	0.2129	16.19
Right gastrocnemius	1.0850	0.1717	15.82
Left tibialis	0.6664	0.1083	16.27
Right tibialis	0.7526	0.1201	15.96

grams) a single gastrocnemius sufficed for a whole series of samples. It will be seen that the per cent dry weight, and therefore the water content, of different samples from the same muscle was remarkably constant. The muscles on the right side, however, appeared in every case to contain slightly more water than those on the left, the differences amounting to approximately 1.5 per cent.

In our first weighings of samples of frozen muscle, the powder was transferred in scoopfuls from the mortar to the weighing bottles which were immersed in ice and salt less than a foot away. The lids were immediately placed on the bottles and a weighing taken at once. It was found difficult to get accurate weights of the cold samples on account of the moisture which collected so rapidly on the surface of the bottles. With

constant wiping, however, consistent weights were obtained, but naturally during this time the temperature within the bottle was rising rapidly. Accurate weighing was also no doubt rendered more difficult by the presence of convection currents set up from the cool surface of the bottles within the balance case. Another weighing was made about half an hour later when the samples had come to room temperature, the lids being undisturbed meanwhile. The results of both weighings are given in table 2. It will be seen that the per cent dry weight remains at about the same general level so long as the weight of the samples remains above 300 mgm. There is, however, considerable variation in the per cent dry weight of samples having approximately the same wet weight. In this respect the frozen muscle shows marked contrast to unfrozen muscle. In samples weighing less than 300 mgm. the per cent dry weight increases with the

TABLE 2

Frog muscle frozen and powdered

SAMPLE	COLD WET WEIGHT	ROOM TEMPERATURE WET WEIGHT	DRY WEIGHT	ON BASIS OF COLD WET WEIGHT		ON BASIS OF ROOM TEMPERATURE WEIGHT	
				Per cent dry weight	Per cent loss water	Per cent dry weight	Per cent loss water
8	3.0479	3.0370	0.4942	16.21	2.34	16.27	2.70
B	2.8798	2.8711	0.4743	16.47	3.90	16.52	4.20
9	1.6991	1.6900	0.2791	16.42	3.60	16.51	4.14
10	0.9567	0.9505	0.1527	15.97	.90	16.06	1.44
H	0.7244	0.7168	0.1178	16.26	2.64	16.57	4.50
11	0.5578	0.5467	0.0916	16.43	3.66	16.75	5.99
12s	0.3056	0.2994	0.0496	16.23	2.46	16.57	4.50
18s	0.2064	0.1984	0.0339	16.42	3.60	17.09	7.62
12L	0.1152	0.1089	0.0200	17.36	9.24	18.36	15.24

diminishing size of the sample. But this experiment, instead of proving a verification of our hypothesis that the larger samples had taken on more water than the smaller ones, showed that the hypothesis was fundamentally wrong. The dry weight of a sample of this particular muscle untreated with liquid air was found to be 15.82 per cent. From the table it will be evident that every sample of the frozen powder had a greater per cent of dry weight than this; consequently instead of the samples having taken on water during freezing, they had on the contrary lost water, the smaller samples to a greater degree than the larger ones.

Comparison in table 2 of the per cent dry weight when calculated from the cold wet weights and room temperature wet weights, shows that loss of water is much greater in the latter than the former. Thus while for a large sample of 3 grams the loss in one case is 2.3 per cent and in the other 2.7 per cent, for a sample weighing 100 mgm., the loss is respectively 9.2

per cent and 15.2 per cent. This great difference may have been partly due to the amount of moisture on the bottles which defied wiping, and to convection currents in the balance case, but it is more probable that although the lids of the weighing bottles were left apparently tightly closed, the expansion of air within them as the temperature rose caused the escape of a certain volume of air, which having been in contact with the powdered muscle would be saturated with water vapor or nearly so. The amount of water which could be lost in this way would naturally be less at the time the cold weighings were made than after room temperature had been

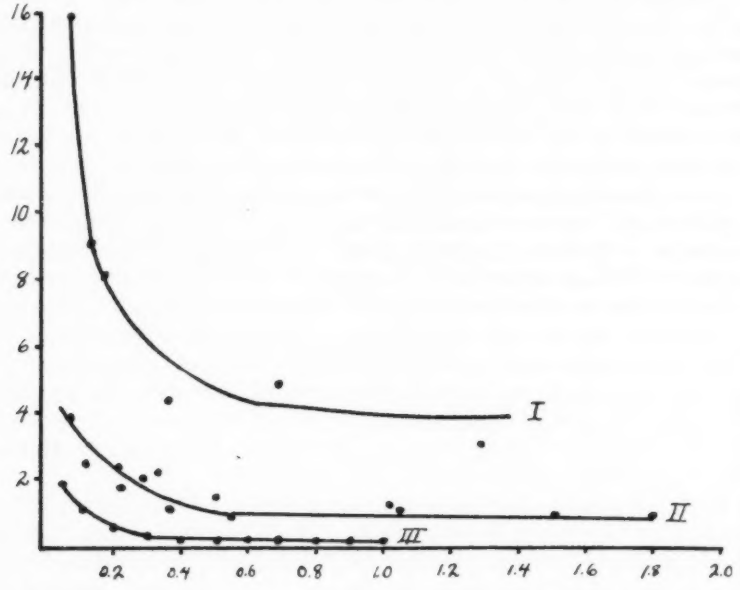

Fig. 1. Ordinates = per cent water loss. Abscissae = grams. Curve I = powdered frozen muscle. Curve II = whole pieces of frozen muscle. Curve III = theoretical loss from closed bottles upon coming to room temperature.

reached. The volume of the bottles was 25 cc. The temperature of the air inside the bottle after the lid was in place might have been as low as 0°C., but hardly lower, since frost was almost never seen inside the bottle by the time weighing was begun. In rising from 0° to 20°C. the air would expand approximately 1 cc. If this cubic centimeter of air, saturated with water vapor escaped, approximately 1 mgm. of water would be lost. If a curve is constructed with weights ranging from 1 gram to 50 mgm. as abscissae, and as ordinates per cent loss in weight when 1 mgm. is subtracted from each weight (curve 3, fig. 1), the break in the curve occurs in the neigh-

borhood of 300 mgm. It appears, therefore, that water was lost not only while the frozen powder was in the mortar and in transit to the bottles, but also from the bottles as well.

Unfrozen samples, on the contrary, weighed immediately after removal from the body, and again an hour later, the lids of the weighing bottles in the meanwhile being left undisturbed, showed no difference whatever in weight.

The effect of the distance through which the powder was carried was determined by placing certain of the weighing bottles directly beside the mortar, and others two meters away. Wet and dry weights were found as usual. In every sample there was loss of water, but approximately twice as much was lost in the samples transported to the distant bottles. Two samples in each set weighed more than 300 mgm. and the loss for each in the near bottle was 1.5 per cent and for the far bottle 2.3 per cent. The third sample in each set weighed approximately 200 mgm; the loss in the near bottle was 3.5 per cent, and in the distant bottle 6.0 per cent.

In another experiment the effect of heaping and spreading the frozen powder on the bottom of the bottle was tried. There was no significant difference in the loss of water in these two methods of depositing the powder. This was perhaps to be expected since the muscle was only briefly exposed to air, and the length of time the sample remained in the closed bottle was the same in each case. But in a similar experiment in which the covers were left off for 2 minutes after the sample was introduced into the bottle there was a profound difference. For a sample weighing 167 mgm., the powder being heaped, the loss in water was 8.9 per cent, but for a sample weighing 169 mgm., the powder being spread, the loss was 17.7 per cent.

It seemed possible that the powdered state might be responsible for this marked loss of water in frozen muscle. Fifteen pieces of muscle ranging from 2 grams to 50 mgm. were immersed individually in liquid air for 30 seconds until they were thoroughly frozen. Each was then replaced in its bottle and cold and room temperature weighings made. All fifteen samples showed loss in water, but the loss was far less than when the muscle was powdered. Approximately 1 per cent was lost in each case on the basis of the cold weight. On the basis of the room temperature weight, approximately 1 per cent was lost for all samples weighing more than 300 mgm. but below this amount the loss rose gradually to 4 per cent for a sample of 100 mgm. Figure 1 shows the difference in loss of water from powdered and whole pieces of frozen muscle, and the theoretical loss due to the expansion of air in the closed bottle with rise to room temperature.

One further point was strikingly brought out in a series of some 25 samples taken from 5 different frogs, viz., the difference in the loss of water

depending on the freshness of the muscle. In this experiment one muscle from each frog was frozen as soon as it was removed from the body and two or three samples taken. The other muscle was removed at the same time as its fellow, especial care being taken with the dissection so as to injure it as little as possible, but this muscle was placed in a closed weighing bottle for 1 hour before being frozen. The usual and consistent loss of water was found in every case for the muscle frozen at once, but in only one case where the freezing was delayed was there loss in water. In eight cases there was no change, and in three a slight gain. Muscles detached from the body slowly accumulate lactic acid on their way to rigor mortis. In the presence of acid, protein gel tends to swell and absorb water. This probably would account for the failure of the muscles standing some time out of the body to give up water on freezing.

CONCLUSION

These experiments show that small samples of freshly removed frog muscle lose weight through loss in water when frozen in liquid air. The water loss is increased upon powdering the muscle, upon transferring it through the air from mortar to weighing bottle, upon allowing it to stand at room temperature even in apparently tightly closed weighing bottles, and upon delay in putting the lid of the weighing bottle in place.

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STUDIES ON THE UTERUS

I. A Method for Recording Uterine Activity in Chronic Experiments on Unanesthetized Animals

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Up to the present, there have been no adequate methods of obtaining records of uterine activity in chronic experiments on unanesthetized animals. Wysenbeek (1), and Wysenbeek and Grevenstuck (2) directly observed the movements of the uterus through a transparent window favorably situated in the abdominal wall of the rabbit, while more recently Templeton and Bollens (3) have attempted to record uterine contractions by means of a balloon placed around a partially exposed portion of the organ, in the dog.

An important approach to this problem was made by Amantea and Krzyszkowsky (4) when they described operative procedures for five types of uterine fistulae in the bitch. It was intended that the physiology of the gravid uterus might be studied in such preparations. Three subsequent papers by Amantea (5, 6, 7) serve to elaborate on the description of the technic, but give no data of experimental attempts to use the fistulae. It may be mentioned that the present work had been in progress for four months when this earlier work came to the attention of the writer.

Methods. *A. Animal preparation:* Under ether anesthesia, the vagina of a non-pregnant rabbit doe is exposed through a mid-line incision, and transected 1½ cm. below the uterine junction with the vagina (see fig. 1A). Thus the uterus, along with the proximal 1½ cm. of the vagina, is separated from the lower end of the genital tract. A blind pouch is made of the remaining vagina, using a continuous suture; it is finally secured retroperitoneally with a few loose stitches. The proximal vaginal sheath along with the ends of the uteri are passed through a stab incision in the lower left abdomen, and allowed to project slightly more than a centimeter beyond the abdominal muscles. It is desirable to make a single stitch into the serosa of one horn where it passes through the abdominal wall, and to secure it to the oblique muscles. After closing the incision loosely about

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the uteri, the vaginal sheath is reflected back over the projecting ends of the uteri, and secured to the skin by six or eight interrupted sutures. Having ascertained the position of the left and right uteri respectively, as they pass through the wall of the abdomen, for convenience in later experimentation, the median incision is closed (see fig. 1B). It has been found well not to use the animals so prepared for ten days or two weeks.

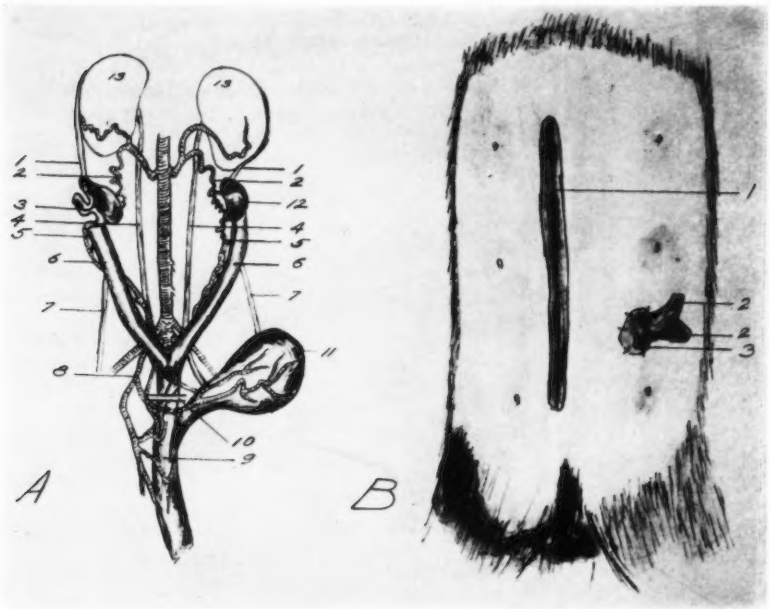

Fig. 1. A, diagram of the relations of the uterus: *1,1* edges of broad ligament. *2,2* ovarian arteries, *3*, Fallopian tube. *4,4* ureters. *5,5* uterine arteries. *6,6* uteri. *7,7* round ligament. *8*, hypogastric artery. *9*, vagina. *10*, operative transection of the vagina. *11*, bladder. *12*, ovary. *13,13* kidneys.

B, surface of abdomen after operation. *1*, line of incision. *2, 2* uterine orifices. *3*, reflected vaginal sheath sutured to skin.

B. Recording apparatus: The uterine activity is recorded by an intrauterine balloon connected with a Brodie bellows through a water and air transmitting system (see fig. 2).

Balloons: Satisfactory recording balloons are made by dipping a pear-shaped glass mold (2 cm. long, 1.2 cm. maximum diameter) on a long handle (3 mm. external diameter glass tubing) into a good grade of rubber cement, the excess rubber being allowed to flow evenly over the surface by rotating the mold at various angles. It is

then vulcanized in the vapor of sulfurchloride. Care must be exercised not to carry this process too far. This may be avoided by frequent removal of the preparation from the vapor, and gently pushing back with the finger the margin of the rubber which is on the handle. When this can be rolled back to the bulb without sticking to one's finger the rubber is sufficiently vulcanized, or very nearly so. A half-minute to a minute is generally enough. The mold is now permitted to stand in the air for several minutes. At the end of this time the balloon should be lightly coated with talcum powder, as should be the fingers of the manipulator also. With a little practice it will be found a simple matter to roll the new balloon off over the bulb, bringing into contact with talcum powder each new surface of rubber as it appears. The balloon should now be sealed to a fire-polished piece of 3 mm. glass tubing 8 to 10 cm. long with hard drying collodium. This should be allowed to stand a short while in a dry place. Once the seal has been effected, the balloon should be kept wet thereafter.

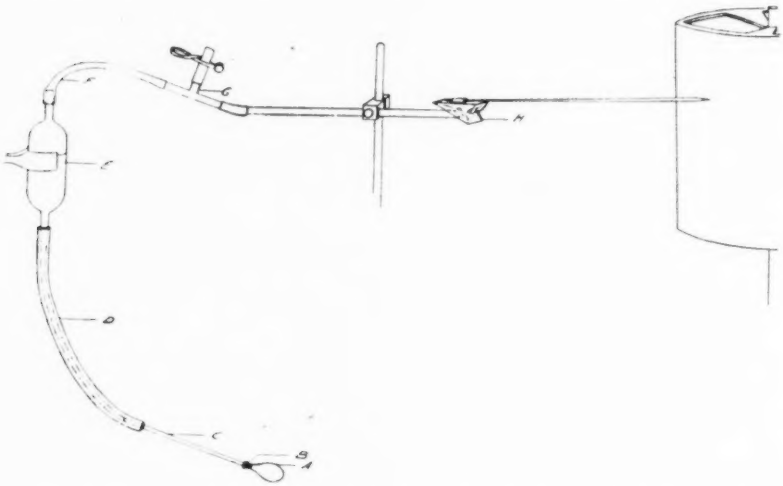

Fig. 2. Diagram of apparatus. *A*, balloon; *B*, collodium seal; *C*, 3 mm. glass tubing; *D*, pressure tubing; *E*, reservoir; *F*, flexible rubber tubing; *G*, T-tube and clamp on stativ; *H*, Brodie bellows.

Reservoir: The balloon so fixed is connected to a reservoir by 15 to 20 cm. of pressure tubing. The reservoir adopted by us is a glass chamber 7 cm. long with an external diameter of 3 cm. (see fig. 2 E).

To fill the balloon, a small amount of water is admitted into the reservoir. By compressing the balloon rapidly, but gently, the air may be displaced by the water in a few minutes. The reservoir may then be filled to the desired height. One-half to two-thirds full has been satisfactory in our work.

Bellows: (see fig. 2 H). A brass plate 2.5 × 1.3 cm. is fixed at an angle of 30° to a brass tube, 20 cm. long, 0.6 cm. diameter. A hole in the center of this plate communicates with the tube. A piece of aluminum of exactly the same size as the brass plate is joined to it by a suitable hinge. A useful hinge is to be had in the thin outside layer of a kid glove peeled off from the thicker leather underneath. This is then

trimmed down to a suitable size and attached to the brass and aluminum plates with (De Kotinsky) cement. The bellows are made of cargile membrane which is fitted to the two plates in the opened position and sealed to them with rubber cement. This is allowed to harden over night. Paraffin may be applied to the upper and lower surfaces of the bellows to make a more effective seal. When soaked with glycerine and water in equal parts the bellows should move freely open and closed, and not leak. Thereafter they should always be kept moist with glycerin and water. A long writing lever of aluminum rolled to an extremely thin point may be secured to a piece of cork which in turn is fastened to the movable aluminum piece of the bellows with paraffin.

A long piece of flexible rubber tubing in which a T-tube is inserted is used to connect the reservoir and bellows (see fig. 2 *F*, *G*). This arrangement enables one to adjust the height of the lever by blowing air into the system, and clamping off the T-tube after the adjustment has been made. With sufficient rubber tubing it is also possible to raise or lower the bellows on a stativ, or to move them from one part of the table to another without affecting the rabbit on the board, or the balloon reservoir system.

Procedure for recording: With the rabbit secured to a board in a supine position, the deflated balloon is eased into the uterine cavity. The balloon should be pulled outward a short distance as the reservoir is raised (25 cm. has been our usual working height). It has been customary in our work to insert the glass tube to which the balloon is attached a distance of 3.5 to 5.0 cm. measured from the exposed end of the uterus. Furthermore, it is desirable to point the glass tube in the direction of the uterus, i.e., dorso-anterolaterally left or right according to which uterus is being used. The distention of the balloon is tested by the application of gentle pressure by blowing by mouth into the reservoir, the meniscus of the water should move slowly down a short distance, and return upon removal of the pressure. The bellows are then connected with the reservoir, and the lever adjusted to a horizontal position by filling the air system as mentioned above. We have found it necessary in our work to surround the reservoir with cotton in order that the air within be kept at a nearly constant temperature.

There is apparently neither pain nor discomfort to the rabbit under the conditions of the experiment. For it is only when there is commotion in the room, sudden noises, or when bothered by flies or touched by people or moving objects that the rabbit pulls at the ties. These times are noted as "struggles" in the protocols.

RESULTS. This method has been applied to the study of the normal uterine activity of several rabbits over a short period of time. The results are briefly noted in table 1.

DISCUSSION. It is obvious from the records we have obtained that uterine contractions are not the only activity recorded by this method. Such extraneous movements as gut peristalsis, struggle movements, micturition, or other intra-abdominal pressure changes may sometimes be seen. These artifacts, however, reveal themselves clearly by characteristic sharp peaks or interruptions of the more leisurely contractions. So, by virtue of their distinctive character, these artifacts are not easily confused with uterine activity.

Intra-abdominal pressure changes, for instance, give abrupt curves that are unmistakable. Examples of this are indicated by small arrows in figure 3 A, B, C, and figure 4 A and C. Micturition gives similar curves

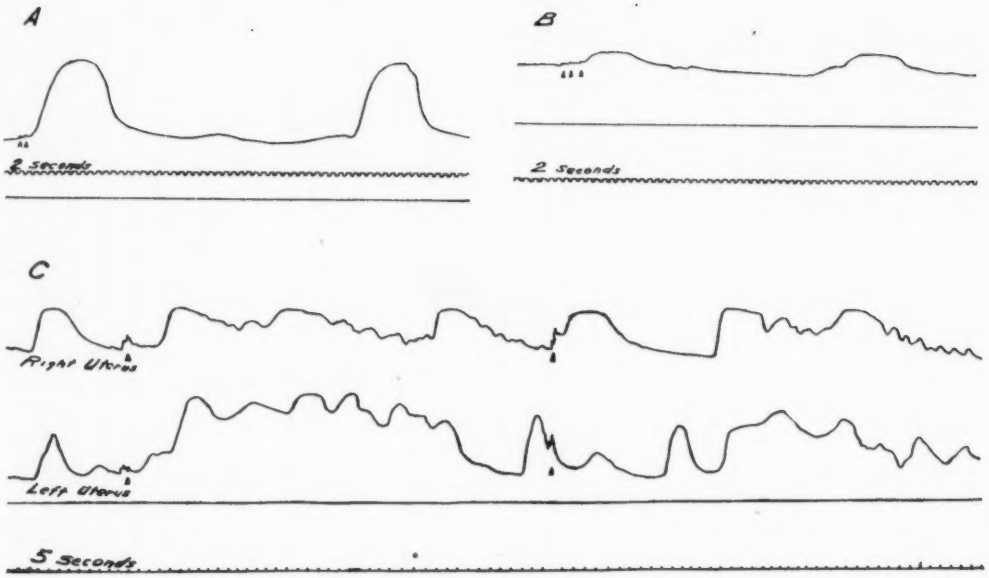

Fig. 3. A, normal uterine activity with balloon under 25 cm. water pressure.
B, same, six minutes later, under 10 cm. water pressure.
C, normal uterine activity, both horns recorded simultaneously; rabbit IV, experiment 7. One-third original size.

TABLE 1

RABBIT	OPERATED	EXPERIMENT	DATE	TYPE OF NORMAL UTERINE ACTIVITY
I	3/8/29	1	3/22/29	No spontaneous activity. Struggle movements showed well
		2	3/23/29	No spontaneous activity. Struggle movements showed well
		3	3/27/29	Short small undulations. Struggle movements showed well
		4	3/29/29	Accepted coitus. Small, irregular contractions before. Large rhythmic after
II	4/6/29	1	4/11/29	Small, irregular undulations (see fig. 4 A)
		2	4/14/29	Small, regular, even contractions, ½ minute intervals
		3	4/16/29	Irregular, but good activity (see fig. 4 B)
		4	4/21/29	Very active: large 2 to 5 peaked contractions (see fig. 4 C)
		5	4/23/29	Irregular, but good activity (see fig. 4 D)
		6	4/25/29	Very large contractions of varying size and duration (see fig. 4 E)
		7	4/28/29	Accepted coitus: good activity before, large rhythmic contractions after
		8	6/21/29	No activity
		9	6/22/29	Almost no activity (see fig. 4 F)
		10	6/24/29	Slight, irregular activity (see fig. 4 G)
III	4/27/29	1	5/14/29	Large, even, regular contractions (see fig. 5 A)
		2	5/16/29	Large, even, regular contractions
		3	5/18/29	Large, fairly even, regular contractions
		4	5/20/29	Large, even contractions, 1 minute apart (see fig. 5 B)
		5	5/23/29	Very large, even, regular contractions (see fig. 5C)
		6	6/26/29	Small, irregular contractions (see fig. 5 D)
		7	6/27/29	Almost no activity. Good response to abdominal pressure, showing system recorded freely
		8	6/30/29	Simultaneous record from each uterus: Right, low undulatory movements. Left, irregular activity (see fig. 5 E)
IV	5/3/29	1	6/21/29	No activity; recorded struggle movements freely
		2	6/22/29	Small, irregular contractions
		3	6/23/29	Slight activity. Good response to pressure applied exteriorly
		4	6/24/29	Large, regular normal contractions every ½ minute for 15 seconds duration
		5	6/25/29	Large, regular contractions
		6	6/27/29	Large, even contractions, at 1½ minute intervals, except occasionally several in succession

TABLE 1—*Concluded*

RABBIT	OPERATED	EXPERIMENT	DATE	TYPE OF NORMAL UTERINE ACTIVITY
IV	5/3/29	7	6/29/29	Simultaneous records from each uterus. Both active, and irregular at first. Right became rhythmical, left remained irregular (see fig. 3C)
		8	6/30/29	Simultaneous records from each uterus: right, large rhythmical contractions; left, irregular, but very active

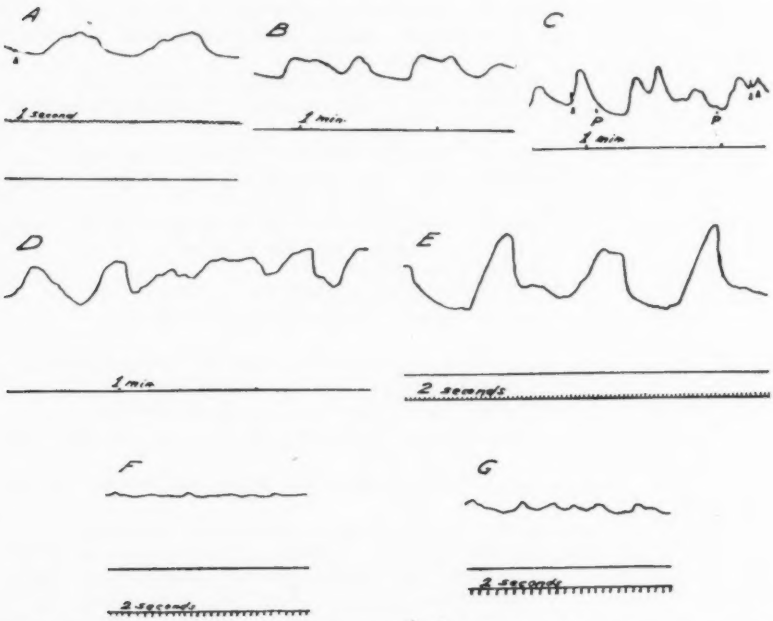

Fig. 4. Normal uterine activity, rabbit II. A, experiment 1; B, experiment 3; C, experiment 4; D, experiment 5; E, experiment 6; F, experiment 9; G, experiment 10. For dates, see table 1. One-third original size.

but of longer duration. Another type of abdominal pressure may at times be seen. Thus, when the uterus is apparently in a low state of tonus, small waves synchronous with respiration are seen on the tracing. Effects of gut peristalsis differ from struggle activities and are at times more troublesome. By correlating the gut movements, however, as seen on the surface of the abdomen, with the tracing, some movements may at times be seen to be associated with small curves on the record. But at other

times the peristalsis which is obvious from the abdominal surface movements, does not show any curves occurring on the record at the same time. The points marked *P* in figure 4 C indicate times at which these abdominal surface movements were seen.

One might raise the objection that even uterine contractions recorded by this method are artifacts, insofar as they are provoked by a foreign body, namely, the balloon. It is true that variations in the tension at which the balloon is distended modify the records obtained. With other

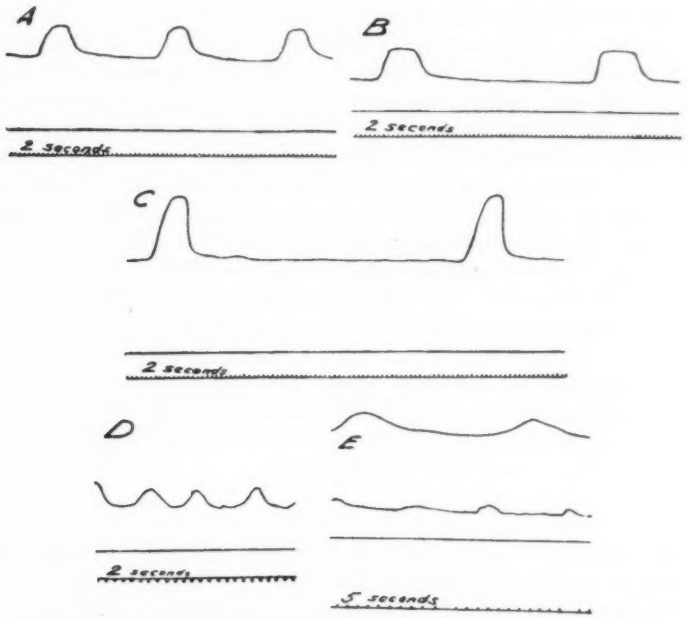

Fig. 5. Normal uterine activity, rabbit III. A, experiment 1; B, experiment 4; C, experiment 5; D, experiment 6; E, experiment 8; top, right uterus; bottom, left uterus. For dates, see table 1. One-third original size.

conditions constant, more vigorous contractions are recorded when the balloon tension is high (compare fig. 3 A and B), than when it is low. Nevertheless there are times when the uterus does not respond to the presence of the balloon regardless of the tensions applied by us. One may justifiably say, therefore, that on such days the uterus is less active or less responsive than on those days when with the identical balloon tension, vigorous contractions are recorded.

It is not easy to account for the variations in uterine activity which are

especially marked in rabbit II and III in which the period of observation was approximately eleven and seven weeks respectively (see figs. 4 and 5). It might be regarded by some that the second period of inactivity represents atrophy of the genital tract. Indeed such might be the case if the uterus became completely inactive. But during the length of time that our observations have been made there is no indication of this. Quite to the contrary, the inactive period in rabbit II did not persist, for activity though slight, was again noted (compare fig. 4 F and G), whereas in rabbit III the inactive period is merely relatively so, contrasted with the preceding very active condition of the uterus (cf. fig. 5 A-E).

Post-operative trauma may produce some change. This was believed possible in rabbit II in which the time elapsing between the date of operation and the first experimental run was five days. But in this case, post-operative trauma can hardly account for the subsequent period of inactivity after marked activity and the acceptance of coitus. It should be mentioned, however, that the extent to which injury to the nerves supplying the uterus is involved has not been fully determined. Yet, it is known that a branch of the hypogastric nerve on each side passes intact to the uteri, along with the uterine arteries, while a lower branch to the vagina and lower uterus is severed. What effect this may have on contractions upon a balloon placed in the tubal end of the uterus is not known at the present time.

Since this factor in the preparations is constant, another factor seems more probable, namely, that of variations in the sex cycle of the animals modifying the activity. The above data are suggestive but cannot be regarded as evidence that such is the case.

SUMMARY

1. A method has been described by which it is possible to record uterine activity in an unanesthetized chronic rabbit. It is possible to record the activity from either horn singly, or from both horns simultaneously.

2. Some data are given on the normal uterine activity of four non-pregnant rabbits, in which the differences in the type of activity in the same rabbit are noted at different times, but the reason for these differences is not explained.

The writer wishes to express his indebtedness and thanks to Dr. Maurice H. Friedman, at whose suggestion this problem was undertaken, and under whose guidance it has been carried out at all times. The writer is also indebted to Mr. W. S. Root who was associated with the work in the early experiments; and to Dr. A. E. Livingston for advice and assistance in exceedingly important details of the method finally adopted for recording. The method of making the Brodie bellows and balloons is that used in the Department of Pharmacology in this University.

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STUDIES ON THE UTERUS

II. Responses of the Non-Gravid Uterus of the Unanesthetized Rabbit to Pituitrin and Pitocin[1]

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Despite the many studies of pituitary extracts on the excised uterus, there have been no records of the effect of pituitrin, and the more recent pitocin, on the uterus (*in situ*) of the unanesthetized rabbit. Therefore these drugs were used on the quiescent or spontaneously contracting uterine pouch.

Experimental procedure. Frequent experiments have been made on eight non-pregnant rabbits over periods of time ranging from several days to eleven weeks. Uterine activity was recorded from uterine fistulae on unanesthetized rabbits by the method recently described by Reynolds (1). The drugs employed were pituitrin "O" and pitocin, generously supplied by Parke, Davis & Co. in 1 cc. ampoules of 10 units, U.S.P., per cubic centimeter. Dilutions of these were made daily of one part of the drug to four parts of physiological saline. Injections were made into any one of the large ear veins, with a tuberculin syringe. When the two drugs were injected into a rabbit on the same day an equal quantity of each was injected, and an interval of three quarters of an hour or more between the two doses was allowed to elapse.

Results. 1. The response of the uterus to intravenous injection of either pituitrin or pitocin depends upon the degree of spontaneous activity of the uterus already existing before the injection. Thus, it is invariably found that when there are spontaneous contractions of large amplitude, long duration, or rhythmical regularity occurring in the uterus, the response to either drug is, accordingly, marked: conversely, when the uterus is almost or completely quiescent the effects are less marked or even nil. (See fig. 1.)

[1] Pitocin, formerly known as oxytocin, is the oxytocic principle of pituitrin, recently isolated and described by Kamm, Aldrich, Grote, Rowe, and Bugbee, Journ. Amer. Chem. Soc., 1928, l, 573.

[2] Hannah A. Leedom Travelling Fellow, Swarthmore College, 1928-1929.

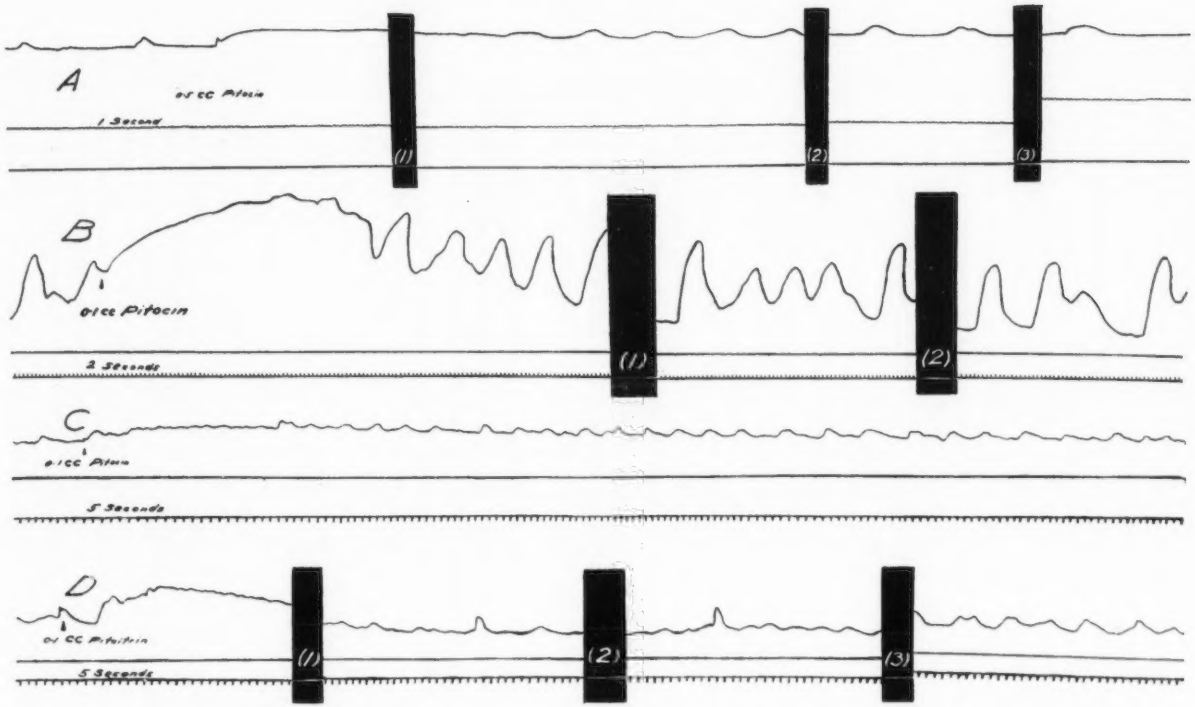

Fig. 1. Effect of intravenous injection of pituitrin and pitocin on the non-gravid quiescent and active uterine fistula. Rabbit 2. Operated upon April 6, 1929. A. April 11, 1929; 0.5 cc. pitocin at a time when the uterus is relatively quiescent. (*1*) 4 minute interval; (*2*) 4 minute interval; (*3*) 15 minute interval. B. April 25, 1929; 0.1 cc. pitocin at a time when the uterus is active. Rabbit had sterile coitus three days later. (*1*) 4 minute interval; (*2*) 15 minute interval. C. June 22, 1929; 0.1 cc. pitocin. D. June 24, 1929; 0.1 cc. pituitrin. (*1*) 1 minute interval; (*2*) 3 minute interval; (*3*) 4 minute interval.

One-third original size.

2. When pituitrin is injected intravenously in rabbit preparations whose uteri are very active, a typical response is almost invariably obtained. There is an immediate tetanus, frequently lasting in our experiments for three to five minutes. Following this there is a gradual relaxation into a period of almost complete inactivity, after which there is a more or less rapid return to the normal type of spontaneous activity. Frequently a decrease in tone persists. (See fig. 1 d; also fig. 2 a and d.)

Three exceptions, however, to this order of events have been observed. In two instances the initial tetanus and period of quiescence were lacking. In one of these the injection of pituitrin was made only thirty-three minutes after a previous injection of pitocin. In this case a lengthening of the duration of the contractions was obtained. The other case was characterized by a marked increase in the rhythm of the contractions immediately following the injections. The third exception was seen while recording simultaneously from both uteri. One uterus gave the response typical of pituitrin described above, whereas in the other, the response was typical of pitocin, described below.

3. The response of the uterus to pitocin, when obtained from very active uteri, in all our experiments was as follows; an initial tetanus indistinguishable from that of pituitrin was obtained, followed *not* by a period of inactivity, but by a period in which the contractions resembled those seen before the injection (see fig. 2 b and c). The period of inactivity seen after pituitrin never occurred in forty experiments with pitocin.

4. The contractions which follow the initial tetanus after the injection of pitocin, and which follow the period of quiescence after pituitrin resemble, as mentioned above, the contractions that existed prior to administration of the drugs. There is seldom an increase in the height of the contraction. The most constant effect is an increase in the *duration* of these contractions as well as a shortening of the interval between them. (See fig. 1 a, c, d; fig. 2 a, b, c, d.)

Fig. 2. Effect of intravenous injection of pituitrin and pitocin on the non-gravid uterine fistula. Rabbit 4. Operated upon May 3, 1929. A. June 24, 1929; 0.1 cc. pituitrin, 2:37 p.m. (*1*), 6 minute interval. B. Same day; 0.1 cc. pitocin 3:39 p.m. The height, duration, and frequency of the two contractions before the administration of pitocin show the recovery one hour after pituitrin. C. June 25, 1929; 0.1 cc. pitocin, 2:08 p.m. The marked increase in duration and frequency were continued for approximately one-half hour. D. Same day; 0.1 cc. pituitrin, 3:32 p.m. (*1*) 6 minute interval. The return to normal frequency is shown before pituitrin injection, while an increase in duration and height persist, with a decrease in tone. The period of marked inactivity shows well after the initial tetanus following pituitrin administration.

One-third original size.

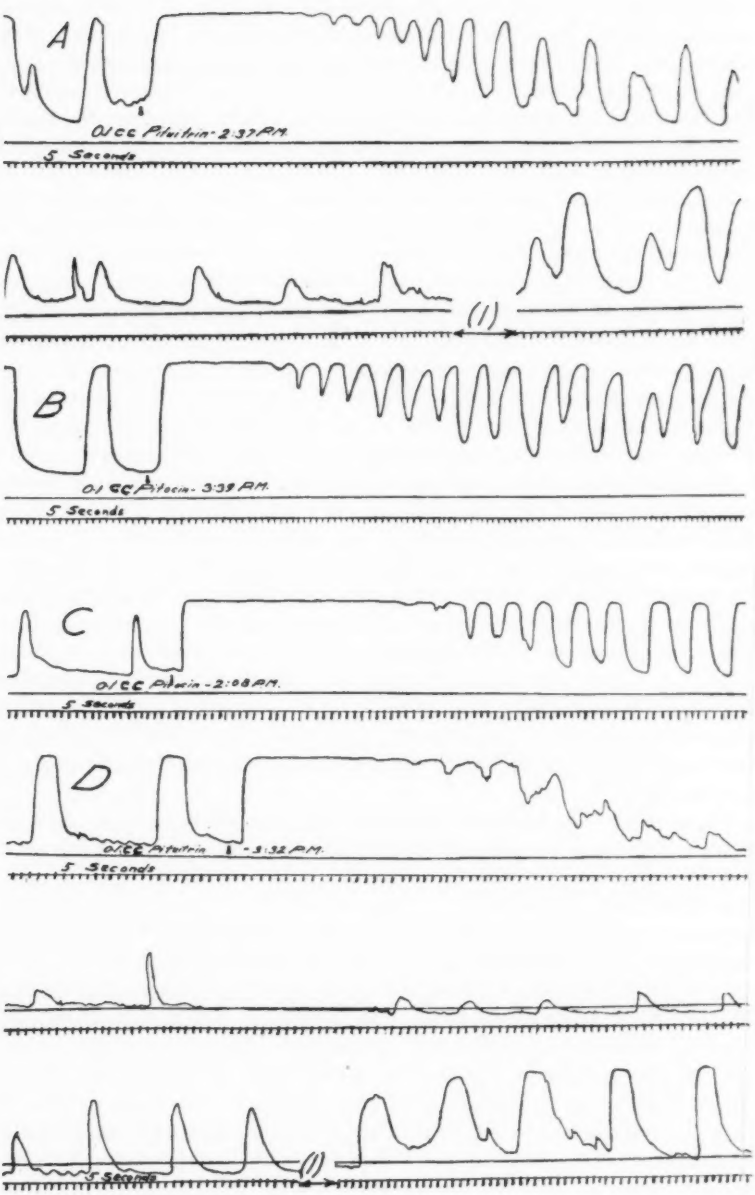
A
0.1 cc Pituitrin - 2:37 P.M.
5 Seconds
B
0.1 cc Pitocin - 3:39 P.M.
5 Seconds
C
0.1 cc Pitocin - 2:08 P.M.
5 Seconds
D
0.1 cc Pituitrin - 3:32 P.M.
5 Seconds
5 Seconds

Fig. 2

5. The amount of the drug injected and the duration of its effect, taking the duration of the initial tetanus as a criterion, do not appear to be correlated. Thus the length of time that this tetanus has lasted, ranges from one to seven minutes with doses of 0.05 to 0.1 cc. of pitocin, and from four to seven minutes with 1 cc. of pitocin. With pituitrin the initial tetanus has ranged from one to four minutes with 0.5 cc., and one to three minutes with 0.1 cc.

6. It was pointed out above that the uterus does not respond to pituitrin or pitocin unless a marked degree of spontaneous activity on the part of the organ is normally present. The question arises therefore whether the failure of response to these drugs in the absence of such activity may not be associated with uterine atrophy. The two following instances may be cited which make this seem doubtful. The uterus of one rabbit (see fig. 1), having shown satisfactory contractions, and responses typical of these drugs for a period of four weeks, showed inactivity of the uterus for a period of five weeks following sterile coitus. Near the end of this time there was virtually no response to pituitrin or pitocin. Then, within the space of a few days responses were obtained which, though small in size, were typical of the active period preceding coitus. It will be noticed in figure 1 also, that a period of relative inactivity occurred before the time when the largest contractions and best responses to these drugs were obtained. In the second case the uterus had shown large contractions and the typical response to pituitrin and pitocin for several weeks. Four weeks later there was almost complete inactivity and no response to the drugs when, as above, within several days a faint but definite response was obtained from the administration of pituitrin. This, then, would seem to indicate that the failure of the uterus to respond to the drugs when it is not spontaneously active should not be attributed to uterine atrophy and correlated dysfunction.

Discussion: As far as we have been able to discover there are but two papers (2), (3) which deal directly with the difference in the response of the uterus to hypophyseal extracts, dependent upon the existing spontaneous activity of the uterus. These investigators have shown that good responses are obtained in the excised uterus regardless of whether it is relatively quiescent or very active. Nor is there any reason to doubt that the excised non-pregnant uterus always responds to the administration of the usual fresh preparations of pituitary extracts. The present pharmacopoeial method of standardization, in fact, calls for the use of the excised uterus of the guinea pig at a time when it is relatively inactive (4), (5) (6). There is also ample evidence that this is true, in the great number of earlier investigations in this field (Dale, 7; Frankl-Hochwart and Froelich, 8; Gunn, 9; and many others). The results which we have described therefore (see fig. 1) in the uterine fistula are not as one might expect from

this earlier work. And although we regard, for reasons mentioned above and elsewhere (1), that it is unlikely that uterine atrophy may be responsible for this type of activity, yet it must be borne in mind as a possible factor.

Nor, to our knowledge, has there been recorded in the literature any account of the period of inactivity regularly following the use of pituitrin on the uterus. Since, then, such a response is almost invariably obtained in the non-gravid uterus, it would be exceedingly interesting to know if the same phenomenon were true for the gravid uterus.

SUMMARY

In the non-gravid uterus (in situ) of the unanesthetized rabbit, it has been observed that:

1. The response to pituitrin or pitocin depends upon the degree of spontaneous activity existing prior to the injection of the drugs.

2. The response to pituitrin or pitocin, when obtained from an active uterus, is characterized by an immediate initial tetanus. Following this, in the case of pituitrin there is a period of relative inactivity lasting for a variable length of time. In the case of pitocin marked, or accelerated activity is resumed immediately after the initial tetanus.

The writer wishes to express his appreciation to Dr. Maurice H. Friedman for his interest and valuable advice. Also to Prof. A. J. Carlson for the generous use of the laboratories and excellent facilities at the University of Chicago where the latter part of this work was done.

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THE HEART RATE OF DOGS BREATHING NORMAL AND OXYGEN-RICH AIR

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Does a dog's heart beat slower when he breathes oxygen-rich air than when he breathes ordinary room air?

This study was suggested by Dr. Thorne M. Carpenter of the Nutrition Laboratory. While visiting our laboratory in the autumn of 1927, he noted that we were accustomed to take only one plus reading on a dog and that at the end of the basal metabolism test in order to avoid any danger of disturbing the animal during the test. Doctor Carpenter suggested that the findings of Benedict and Higgins (1911) on human beings would indicate that the heart rate after the run was not an accurate picture of the heart's condition during a test, if we used a bell filled with pure oxygen, since these workers found: "that the pulse rate is lower with oxygen-rich air than when breathing ordinary air; furthermore, that the higher the percentage of oxygen breathed (up to 90 per cent), the lower the pulse." We immediately decided to check these findings on three animals then under careful observation.

EXPERIMENTAL METHOD. The dogs used had been given daily metabolism tests for a period of twenty months and were, therefore, perfectly adjusted to all kinds of handling relating to testing. We merely began counting the pulse three times instead of once. We decided to count the pulse, first, just before the metabolism test after the dog had rested and breathed room air for from forty to one hundred twenty minutes; secondly, just before the end of the metabolism run, after the dog had breathed an oxygen-rich atmosphere for fourteen minutes; and thirdly, after the test and after the dog had returned to breathing room air for ten minutes or longer. All pulse counts were made by palpation of the left chest with the dog lying on his left side. The count was made after successive fifteen second trials showed no differences. Between November 1927 and June 1928, we secured 1,289 tests giving these data.

THE DATA AND THEIR TREATMENT. If one determines all possible combinations of series of three numbers on the basis of whether they are equal, greater, or less than each other, one finds nine possible combinations.

Thus they may all be equal; the first and second may be equal and less or greater than the third; the second and third may be equal and less than the first; the second may be less than both the first and third and so on. To these nine types, more clearly characterized in the table, we arbitrarily assigned numbers from I to IX. We next classified our 1,289 groups of three pulse rates under these nine type headings. Each dog's data were classified separately. The accompanying table shows the number and percentage of frequency of occurrence of each type for each dog and for the three combined. There are included also the figures secured by averaging separately the first, second, and third pulse counts of all the tests falling within each type for each dog.

DISCUSSION OF RESULTS. In our work no definite figures are available to show the proportion of oxygen present in the oxygen-rich mixtures.

Table showing series of pulse rates classified according to types

TYPE	DOG 5					DOG 6					DOG 7					ALL DOGS	
	Frequency of this type	Per cent	Averages of pulse rates per minute			Frequency of this type	Per cent	Averages of pulse rates per minute			Frequency of this type	Per cent	Averages of pulse rates per minute			Total frequency	Per cent
			Room air	High oxygen	Room air			Room air	High oxygen	Room air			Room air	High oxygen	Room air		
I	51	12.50	42.9	42.9	42.9	67	16.3	37.6	37.6	37.6	87	18.3	41.2	41.2	41.2	205	15.90
II	43	10.6	40.3	40.3	43.0	58	14.1	36.9	36.9	40.0	40	8.4	41.2	41.2	44.9	141	10.94
III	27	6.6	43.9	43.9	39.7	18	4.4	40.4	40.4	37.6	34	7.1	43.6	43.6	40.9	79	6.13
IV	57	14.07	43.8	41.0	41.0	45	11.0	39.7	36.8	36.8	85	17.8	44.2	41.2	41.2	187	14.50
V	54	13.3	43.8	40.2	44.8	63	15.4	39.0	35.6	39.6	76	16.0	43.0	39.8	43.2	193	14.97
VI	13	4.19	46.0	42.9	40.6	17	4.1	39.0	37.5	35.2	15	3.1	45.0	42.0	40.1	45	3.49
VII	69	17.03	40.7	44.2	44.2	76	18.5	37.0	40.1	40.1	38	8.0	40.6	46.0	46.0	183	14.19
VIII	49	12.0	40.5	44.4	47.7	33	8.06	36.8	39.7	40.8	39	8.2	39.8	41.9	45.3	121	9.39
IX	42	10.3	44.0	48.3	44.8	32	7.82	38.7	42.2	39.2	61	12.4	41.7	44.8	42.1	135	10.47

Our apparatus is the regular Sanborn Benedict portable model with motor blower on the outside. Before each test, the bell is filled from an oxygen tank. From rough calculations, it seems certain that the added oxygen sufficiently dilutes any nitrogen present in the dead air space of the apparatus to keep the oxygen concentration always upwards of 70 per cent. Since from one test to the next the remaining air is often forced out of the instrument without breaking the water seal and replaced with pure oxygen, the percentage of oxygen would increase rather than decrease in a long series of tests.

CONCLUSIONS AND DISCUSSION. Granting that our dogs breathed an oxygen-rich mixture, our data lead to the conclusion that such mixtures do not cause a slowing of the pulse of a thoroughly relaxed dog. Thus a

drop in pulse rate during oxygen breathing with a return to a higher level after resumption of air breathing as was typical of the findings of Benedict and Higgins corresponds to our type V and is found in only 14.97 per cent of our cases; whereas type I, showing no change is found in 15.90 per cent of the cases; and type VII showing an increase in pulse rate on high oxygen is found in 14.19 per cent.

The figures lend themselves to still better forms of comparison. Let us compare the pulses secured while first breathing room air with those secured after fourteen minutes on high oxygen forgetting for the time being the the third pulse rates. Types I, II and III comprise the cases in which there was no change in pulse upon going from room air to high oxygen. Together they contain 425 tests or 32.97 per cent of the total. Types IV, V and VI comprise those tests in which the pulse rate dropped upon taking the oxygen-rich mixture. This group also contains exactly 425 cases. Types VII, VIII and IX, which consist of cases in which the pulse rate increased upon the taking of oxygen-rich mixtures, comprise 429 or 34.06 per cent of the cases. One is forced to conclude that the direction of change, if any, in the pulse rate upon going from room air to high oxygen mixtures is not determined by the oxygen mixtures.

Yet another form of analysis is interesting. If one compares the direction of pulse rate change as the animals go from high oxygen back to room air the conclusion is unaltered. Types I, IV, and VII cover those instances in which the pulse rate did not change as the animal went from oxygen rich to ordinary air. This group contains 575 or 44.61 per cent of all the cases. Types II, V and VIII consisting of cases in which the pulse did increase with the change from high oxygen to room air comprise 455 or 35.29 per cent of the total. Types III, VI and IX together cover cases in which the last pulse rate was actually lower than that on high oxygen mixtures and contain 259 or 20.09 per cent of the cases. It is to be noted that here again only slightly more than one third of the cases (35.29 per cent) show rises after resumption of room air. Since there are only three possible changes, chance alone would provide $33\frac{1}{3}$ per cent in this direction. If the several operations pursuant to the taking of a metabolism test, applying the muzzle, working the valves, and taking three pulse rates has any effect on the dog, one might expect it to be cumulative and, therefore, affect the last reading most. We suggest that this may be the factor responsible for the moderate distortion from what would be purely chance distribution in the findings of this last analysis.

SUMMARY

1. The pulse rates of dogs changing from room air to high oxygen mixtures and back to room air as found in 1,289 distinct tests on three thoroughly trained male dogs are presented and analyzed.

2. The nine types of pulse rate changes theoretically possible are found. Their frequency ranges from 3.49 per cent to 15.9 per cent.

3. There is evidence that the changing from room air to oxygen-rich mixtures and back again to room air is *in no manner related* to the fluctuations in pulse rates found in the thoroughly relaxed resting dog.

4. Incidentally, the data show that the averages of pulse rates of normal dogs under basal conditions lie between 37 and 48 beats per minute.

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THE MALE HORMONE

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Disregarding the older literature, which is voluminous and confusing (1) we may begin by referring to the work of Pézard (2), who examined the masculanising effects of testicular grafts and ascribed them to the presence of a male hormone. Somewhat similar experiments had been made by Steinach (3). Many subsequent attempts to extract the male hormone ended in failure, mainly because no adequate tests were employed to measure the potency of the extracts. To Pézard, also, we owe the next notable advance; for he demonstrated the presence of the male hormone in the blood of cocks by using the castrated cock as a test animal (4). It seemed logical to conclude that since the male hormone is in the blood, it would probably also be found in the urine. The urine of young men was, therefore, our starting material.[2]

In the beginning we used either urine as such (the urine having been first concentrated to a very small volume) or a urine which had been first treated with alcohol and the concentrated filtrate applied. It became obvious, from measurements on the comb and wattles of capons, that such extracts contained the male hormone. It also became apparent, even early in our investigations, that the hormone activity of the urine markedly decreases with age (5).

It was not at all surprising that such extracts as we used in our preliminary work should have had toxic effects; and it soon became necessary to

[1] Preliminary reports have appeared in the Proc. Soc. Exp. Biol. Med., xxvi, 325, 569 (1929), and in the report of the Thirteenth International Physiological Congress, p. 89 (1929).

We are indebted to Mr. A. Heckscher, Mr. Harold McCormick and Mr. Jules S. Bache for making the work possible. We also wish to thank Dr. Harry Benjamin for his co-operation and interest in the work.

[2] In the course of our experiments we also tried testicular extracts, the results of which will be recorded presently. In the meantime, Koch and his collaborators, working on bull's testicles, have presented a report before the International Congress of Physiology, page 148 (1929) which, in many respects, confirms our own work.

After our article had been sent to press, a résumé of the work of Koch and collaborators appeared in the *J. Biol. Chem.*, **84**, 495 (1929) [November].

devise methods for the preparation of a purer and more concentrated product. An observation by Veler and Doisy in connection with their work on the female hormone (6) proved of value. These authors showed that the female hormone could be extracted from the urine of pregnant women by means of chloroform; and this method we now applied to the extraction of the male hormone from the urine of young men. While this procedure eliminated the toxic effects of the urine, and permitted more concentrated extracts to be used, the chloroform extract proved less active than the original urine. This may have been due to the fact that the extraction with chloroform is incomplete (perhaps because the urine was not sufficiently acid); or that the urine contains some other active substance (anterior pituitary?) which is insoluble in chloroform.

In the meantime, we were investigating some chemical characteristics of the female hormone (7) and these results proved of great value in elucidating the properties of the male hormone. It was shown that the female hormone is not present in the unsaponifiable fraction, as was believed, but rather in the fatty acid fraction. The confusion had resulted from the fact that the female hormone forms alkali salts which are largely soluble in ether. Obviously, then, when one saponifies, and extracts the unsaponified portion with ether, much of the hormone is extracted.

It was now shown that what is true of the female hormone is true of the male hormone: both form alkali salts which are largely soluble in ether. It was this information which eventually led to a modified method of extracting the male hormone.

EXPERIMENTAL. The test animals were white leghorn cocks. Castration was performed upon them when they were two months old. In removing the testicle, care was taken not to allow any liquid to trickle out, otherwise there is always danger that a new testicular formation may result.

Our criterion of a successful operation was the immediate response in comb growth upon the injection of the male hormone, and the constant and rapid retrogression of comb size when injections were stopped.[3]

The comb and wattles were measured before castration, and every 15 days after castration. The method of measurement, once adopted, was strictly adhered to throughout all the experiments. It consisted in each instance, in measuring the distance from the highest point to the base line, the vertical line being measured at right angles to the base line.

Within a few days after castration, the animals show a very noticeable retrogression of comb and wattles, and their color changes from a bright red to a pale pink. The comb, after a time, falls to one side and looks shrivelled, and there is a desquamation of the epitheleum. After injecting

[3] We speak here and elsewhere of the "male hormone." All we mean to imply at this stage is that we have an extract which influences the growth of comb and wattles, and this extract, for purposes of convenience, we designate the "male hormone."

the active material, a change is noticed within a few days—at least, after some experience has been gained with the results of the operation and the subsequent treatment. The color begins to brighten. There is a noticeable increase of turgor. The comb and wattles begin to enlarge. As treatment is continued, the animal assumes more and more the appearance of a non-castrated animal. Should the injections be stopped for several days, there is a very marked general retrogression.

To test the activity of our various extracts, then, we applied the criteria just outlined. Each extract was tried on six cocks simultaneously, and the average of these six results was taken. An extract was considered inactive if no noticeable change could be observed in the course of five days.

Selection of a standard for measuring the potency. The importance of having some standard by which the potency of various fractions can be estimated, is obvious. For this purpose we have selected a "cock unit." The "cock unit" is that amount of male hormone which, when injected, will increase the size of comb and wattles to the extent of 10 mm. in 10 days. For each test six animals are used and the average taken.

Some preliminary experiments. In the hope that castration would prove unnecessary, experiments were performed on two-weeks old male and female chickens. These were injected with the equivalent of 1 cock unit per animal per day for 15 days.

	Comb increase *per cent*
Males injected	95
Controls	117
Females injected	70
Controls	47

The negative results with the males made us reject this method of testing.

The effect on comb and wattles of castrated animals is seen in the following examples, the measurements having been begun at the time when the shrivelling process had come to a standstill:

	Increase or decrease in comb and wattles *per cent*
1. Group of six capons observed for 9 weeks	+1
2. Group of six capons observed for 9 weeks	−2
3. Group of six capons observed for 8 weeks	1
4. Group of six capons observed for 8 weeks	0
5. Group of six capons observed for 8 weeks	−2[4]

[4] In these cases, as well as in subsequent examples, the figures represent the average increase or decrease.

It is obvious that, without treatment, and provided the animals are successfully castrated, there is no change in size of comb and wattles.

Relationship of the male and female hormone. Here three groups of animals were used. Two of the groups represented well castrated cocks and the third group represented badly castrated cocks, which, however, up to the time of this experiment, had shown no comb growth. *a.* One group of well-castrated cocks received two rat units of female hormone per cock per day for a period of two weeks. Decrease in comb and wattles, −2 per cent. *b.* One group of well-castrated cocks received three rat units of female hormone per cock per day for a period of three weeks. Decrease in comb and wattles, −3 per cent.

Group *b* now received one cock unit of the male hormone per cock per day for a period of one week. Increase in comb and wattles, +12 per cent.

Group *b* now received one cock unit of the male hormone plus ten rat units of the female hormone per cock per day for a period of one week. Increase in comb and wattles, +4 per cent.

c. One group of badly castrated cocks received three rat units of female hormone per cock per day for two weeks. Increase in comb and wattles, +13 per cent. Growth now continued without any further injection.

As the number of experiments, in this connection, as well as the number of observations, were limited, no definite conclusions should be drawn. It does seem, however, as if in well-castrated animals the male and female hormones may act antagonistically, at least in so far as secondary sex characters are concerned. On the other hand, in badly castrated animals, the female hormone seems to have a stimulating effect on the testicular remnant.

The oral administration of the male hormone compared with its administration in the form of an injection. The advantages of being able to administer the male hormone *per os* are obvious. In the following experiments we examined this possibility.

a. In a preceding paper (8) we have shown that the injection of a chloroform extract of 100 cc. urine per cock per day for 15 days gives rise to an increase in comb and wattles of +28 per cent.

b. Injection of a chloroform extract from 86 cc. urine[5] per cock per day for one week. Increase in comb and wattles, +10 per cent.

c. Injection of 20 cc. urine per cock per day for one week. Increase in comb and wattles, +5 per cent.

d. One hundred cubic centimeters urine given *per os* per cock per day. Percentage increase at the end of the first week, +5; percentage increase at the end of the second week, +8.

e. Experiment *d* was repeated with another group of cocks. Percentage

[5] The exact method of extraction will be given later. Unless otherwise stated, the urine is that obtained from men below 50 years of age.

increase at the end of the first week, +5; percentage increase at the end of the second, +4.

f. Experiment *d* was repeated, but this time a chloroform extract equivalent to 150 cc. urine was given. Percentage decrease at the end of one week, −3.

g. The equivalent of 50 grams of chloroform extract of pig's testicle, incorporated in olive oil[6] was injected into each cock every day for three weeks. Increase in comb and wattles, +14 per cent.

h. The same as *g*, except that an alcoholic extract was made.[7] Increase in comb and wattles in two weeks +9 per cent.

i. Here *f* and *g* were combined and given *per os* for one week. Percentage increase in comb and wattles, +9.

j. Here *f* and *g* were again combined, and given *per os* for one week, but the equivalent of 100 cc. of urine, instead of 150 cc. was used. Percentage increase in comb and wattles, +2.

k. Here *f* and *g* were once more combined and offered *per os* for two weeks, but the equivalent of 200 cc. of urine was incorporated. Percentage increase in comb and wattles, +11.

l. The equivalent of a chloroform extract of 50 grams of the reproductive organs of the pig (testicles, Cowper's gland, prostatic gland, etc.) together with the equivalent of 200 cc. of a chloroform extract of the urine, were given *per os* for one week. Percentage increase in comb and wattles, +6.

It would seem, from the results here recorded, that the activity of the male hormone does not entirely disappear when given by mouth. It also appears that testicular extracts, like urine extracts, are active, though the comparative activities of these two extracts have yet to be determined.

The object of combining testicular with urine extracts was to ascertain, if possible, whether some activating substance other than the male hormone was present in the urine which might, in turn, influence the hormone in the testicle. The results appear negative.

Experiment *l* suggests that the portions of the reproductive organs other than the testicle contribute little, if any, to the hormone activity.

The dependence of the amount of male hormone on the age of the individual. This phase of the problem has already been discussed in two previous publications (9). It seems evident that hormone activity decreases with increasing age. The possible diagnostic applications of such a relationship are matters which we intend to study.[8]

[6] The method of preparation will be given later.

[7] The filtrate from the alcoholic extraction was partially evaporated, and an excess of ether added. This was filtered, the filtrate distilled and the residue dissolved in oil.

[8] In this connection it should be mentioned that we are now investigating whether the anterior pituitary substance is present in the urine of males, and whether, if present, the amount is influenced by age.

Preparation of the male hormone. While the early experiments were carried out by using urine, partly evaporated, further developments were made possible by parallel work on the female hormone. From the work on the female hormone (using the urine of pregnant women, the placenta, the ovaries, etc.) the following conclusions were drawn (10):

The female hormone is sparingly soluble in water, but it combines with alkalis to form alkaline salts which are soluble in water. Hitherto the hormone had been regarded as being present in the unsaponifiable fraction, but this view could not be corroborated. When the material containing the hormone is subjected to saponification and the products are extracted with ether, the hormone is found both in the unsaponified and in the saponified fraction. The alkali salts of the hormone are partially soluble in ether. If, however, chloroform is used instead of ether, we find that the alkali salts of the hormone are insoluble in chloroform, and therefore they appear in the fatty acid fraction.

In the case of urine, saponification is unnecessary. It suffices to add to the urine enough acid to liberate the hormone from its alkaline combination (as an ammonium salt, perhaps?) and then to extract with chloroform, in which solvent the free hormone is soluble. Using this method with tissues does not yield good quantitative results; and here further work is necessary to determine whether a subsequent saponification will be necessary. In any case, whether the starting material be tissues or urine, the procedure is at present the same.

For the time being, what has been said of the female hormone applies to the male hormone. We have used for the most part the urine of males for our source material, though testicles can also be used. The male hormone behaves very much like the female hormone in its chemical properties; and while, undoubtedly, chemical differences will sooner or later be found, at this stage one might well emphasize the chemical similarities. By acidifying the urine, the male hormone can be extracted with chloroform. Further purification is dependent upon the fact that the material can be subjected to steam distillation, thereby getting rid of phenolic impurities, etc., without any resulting loss of activity.

A good yield of the male hormone from urine is only obtained when the urine is made distinctly acid prior to extraction with chloroform. This, unfortunately, has the disadvantage that under such conditions the chloroform dissolves large quantities of urinary coloring materials which are then difficult to remove without a loss of hormone activity. Various other methods are at present under investigation.

The hormone from the testicle. Two procedures were used: a. Fifteen hundred grams of pig's testicle were minced and poured into six times the volume of 95 per cent alcohol. This was filtered and the filtrate evaporated *in vacuo*. The residue was taken up in olive oil in such amounts so that 1 cc. of the oily product corresponded to 50 grams of the fresh testicle.

b. Fifteen hundred grams of pig's testicle were minced, air-dried at 60 to 70°, powdered, and extracted in a Soxhlet with boiling chloroform. The chloroform extract was evaporated *in vacuo*, and the residue—oily in appearance—was used directly. Here, also, 1 cc. of the product corresponded approximately to 50 grams of fresh testicle.

Methods a and b were also used for extracting the entire genital apparatus of the pig.

The hormone from male urine. It is advisable to start with at least 10 liters of urine. This can be preserved, while being collected, with a small amount of thymol. In order that a maximum yield may be obtained, it is essential that the urine be acidified. We acidify it with hydrochloric acid until the urine is acid to congo red paper. Chloroform is used as the extracting material. For 10 liters of urine we use 2 liters of chloroform. The mixture is gently heated under a reflux for about 8 hours. It is advisable, at least once during this process, to be sure that the acidity is maintained by actually testing the mixture.

This simple method of extracting the urine with chloroform works very well. When the boiling point of the chloroform is reached the particles stream through the urine layer, into the condenser, and back again into the urine layer. A second extraction with chloroform is not necessary, since such a fraction shows very little activity.

The product is now cooled and most of the urine (watery layer) carefully poured off. The residue is transferred to a separatory funnel, stirred and allowed to stand for a little while. The chloroform layer is drawn off. The emulsion in between the chloroform layer and the residual urine is now drawn off, filtered (using a Buchner, with gentle suction) and washed with a little chloroform. The filtrate is again separated in a separatory funnel, the chloroform fraction drawn off and added to the main chloroform portion.

The combined chloroform extracts are evaporated in a distilling flask, using a water bath. The chloroform is in this way recovered and used again.

Using the same flask, the residue is next steam distilled. The distillation is continued until no more oil comes over.

Enough sodium hydroxide is now added to make the residue alkaline to phenolphthalein. The mixture is warmed and filtered in the cold. The filtrate is adjusted to a pH of 7.4 and made up to 50 cc. with distilled water.

This gives 1 cc. of the final product equivalent to 200 cc. of the original urine.

In this instance, one cock unit would be obtained from 75 to 100 cc. of urine.

In most of the experiments so far conducted, including the experiments illustrated by the photographs, the stock solution was the one just described.

To illustrate the activity of this stock solution, we may quote the following:

Fig. 1

Six castrated cocks were given daily injections of 0.5 cc. of the solution per cock. This represented the equivalent of 100 cc. urine. The average length of the comb and wattles in the beginning of the experiment was 31 mm. At the end of 26 days the average length had increased to 58 mm., or a percentage increase of 87.

We have found that the hormone (in alkaline combination) can be salted out by salts such as sodium chloride and ammonium sulfate. It also separates out on the addition of strong alkali. Some of the urinary coloring materials can be eliminated by precipitation with acetone and ether.

The accompanying photographs illustrate the changes in comb and wattles as a result of the hormone injections: *1* represents castrated animals (controls); *2* represents animals of the same age as the controls after 8 days' treatment; *3* are the same as *2* after 15 days' treatment; *4* are the same as *2* after 26 days' treatment; *5*, *B* and *D* are the same as *2* after 26 days' treatment, and these are compares with *A* and *C*, which are non-castrated cocks of the same age.

We may add that the castrated cocks represented in *1* look much worse now than they did when the photographs were taken.

We may also add that when the injections are stopped, the cocks rapidly revert to the castrated type.

For example, in photograph *6* we have the treated cocks of the type shown in *5*, in which no injections were administered for 29 days.

SUMMARY

1. The urine of young men contains a hormone (or hormones?) which induces comb growth in capons when a properly prepared extract of such urine is injected.

2. The extracts obtained from testicles, when injected, also give definite, but less marked effects. The difference may depend upon the amount of material used.

3. Administration *per os* also has some effect, but the effect is much less pronounced and less constant than when the extract is injected.

4. The male and female hormones show marked similarity in chemical properties. Both may be extracted with chloroform; both form alkaline salts; both are found in the fatty acid fraction; and the methods of purification so far adopted apply to both hormones.

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Addendum

Since the above has been written we have made some improvements in the method of extracting the hormone. We find it advisable to acidify the urine very strongly indeed. For 10 liters of urine not less than from 500–700 cc. of conc. HCl are used to advantage. We also find that appreciable quantities of impurities may be removed by a double precipitation with ether after the steam distillation process. The hormone is, of course, soluble in ether. The final product can be sterilised without impairing the activity of the material.

EFFECT OF ETHYL ALCOHOL ON THE GROWTH OF CHICKS

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The object of this experiment was to determine the effect of ethyl alcohol of different concentrations on the growth of chickens. No similar work could be found in the literature, although studies have been made on normal growth of chickens, (1), (2), (3), (4).

METHODS. Forty-four day-old white Leghorn chicks, received at the laboratory in good condition on February 8, 1929, were divided into three groups: 17 to receive a strong solution of alcohol, 17 a weak solution and 10 to be given water in the same manner.

The chicks were kept together in an enclosure where they were exposed to direct sunlight; their diet consisted of mash, scratch feed, cabbage, brown bread, alfalfa and clover. At the age of three months the sexes were separated by means of a partition in the pen. Each chick was weighed every Friday afternoon.

The alcohol and water were administered each morning between 7:00 and 7:30 by means of a graduated pipette inserted deep down into the esophagus, the liquid being released into the crop at a moderate rate of speed. If the pipette was not inserted deep enough, or the liquid allowed to run out too rapidly, the material would dam up in the throat and some of it enter the glottis, causing the chick to strangle, sometimes with fatal results.

The light alcohol chicks were given 0.3 cc. of 10 per cent ethyl alcohol solution from February 8 to 15; February 16, 0.5 cc. of 15 per cent alcohol; February 17 to 24, 0.6 cc.; the dose being gradually increased every few days so that by March 6 they were receiving 1.00 cc. of 15 per cent alcohol. By April 1, the dose had been increased to 10 cc., April 9, 12 cc.; April 16, 15 cc.; April 21, 18 cc.; and May 6, 20 cc. From May 18 on they received 25 cc. The control chicks were given equal amounts of water in the same way.

The heavy alcohol chicks were given the same amounts as the light alcohol group until April 1. The first week they received 20 per cent alcohol, but after that 30 per cent. From April 1, the dose given each chick varied according to the amount required to intoxicate it, the dosage

being roughly proportional to the weight of the bird. At the close of the experiment the range varied from 7 to 13 cc.

Unfortunately a number of fatalities occurred in the heavy alcohol group, most of them during the first three weeks. In some cases the alcohol was too strong, in others some of the liquid had entered the bronchial tubes. The chicks moped around for a day or two before succumbing, every now and then gaping for breath.

RESULTS: The chicks given heavy doses of alcohol were more irritable and excitable than the other groups; they appeared less alert, and the

TABLE 1

Average weekly weights

AGE	CONTROLS		LIGHT ALCOHOL		HEAVY ALCOHOL	
	Males	Females	Males	Females	Males	Females
	Number of chicks at end of experiment					
	5	5	11	6	4	3
weeks						
0	36.6	35.96	37.55	34.8	36.5	34.55
1	40.4	39.5	45.93	44.4	44.6	40.0
2	53.8	62.6	64.36	62.0	55.6	52.0
3	81.2	87.3	100.9	97.0	83.0	78.0
4	113.2	125.8	137.1	130.8	108.6	107.3
5	154.2	167.0	184.45	173.6	151.3	146.0
6	209.8	226.2	248.72	234.0	198.6	190.5
7	279.2	277.6	324.63	289.83	247.0	247.8
8	388.2	376.0	429.2	388.0	317.0	348.0
9	463.6	428.0	497.0	443.0	383.0	396.0
10	530.0	476.6	563.0	492.5	444.2	437.5
11	587.2	514.6	650.5	571.3	510.2	495.5
12	704.4	607.0	726.6	635.0	599.0	572.6
13	775.0	702.6	825.2	747.0	670.0	664.6
14	862.4	835.3	922.0	835.3	794.0	745.0
15	937.6	888.4	1009.0	925.0	872.0	811.0
16	954.0	891.0	1023.0	940.0	879.5	832.0

color of their feathers and combs was not so bright. They also drank more water than the others.

The group receiving light doses of alcohol had on the whole, larger, fuller, redder combs and brighter, more compact feathers than either of the other groups. In disposition they did not differ from the controls.

As to growth, throughout the experiment the birds receiving light doses of alcohol led all the others as is shown in the accompanying table: the controls came second, while those receiving heavy doses of alcohol were invariably behind. Even the females of the light alcohol line outgrew the

male controls up to the age of 8 weeks. During the last 8 weeks of the experiment the order according to rapidity of growth was as follows: males of the light alcohol group; male controls; females of the light alcohol group, female controls, males of the heavy alcohol group, females of the same.

SUMMARY

1. The males receiving 15 per cent ethyl alcohol grew fastest of all the chicks. The males receiving equal amounts of water came second.

2. The females receiving 15 per cent alcohol outgrew the females receiving equal doses of water and also both the males and females receiving 30 per cent alcohol.

I am indebted to Prof. L. B. Nice for suggesting this problem and for criticism throughout the work.

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STUDIES ON THE INNERVATION OF SMOOTH MUSCLE

V. On the Relation of Vagal Gastric Effects to Wedensky Inhibition

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Four years ago Veach (1) showed that vagal inhibition of the lower end of the esophagus, cardia, and stomach of the cat was of the nature of Wedensky inhibition. This relationship was found to hold with a variety of methods of preparation of the experimental animals. Recently, however, McSwiney and Wadge (2), working with cats anesthetized with luminal and ether have asserted that vagal effects on the cat's stomach depend only on the degree of tonus of the musculature of the organ preceding stimulation. They maintain that stimulation of the vagus, regardless of frequency or intensity, produces inhibition when the tonus is high and contraction when the tonus is low. Veach (1) recognized in his original paper that high tonus of the stomach favored inhibition and that low tonus favored contraction, and he showed that such reactions were to be expected in view of the similarity of vagal inhibition to Wedensky inhibition. Luminal was not used as an anesthetic in Veach's work, however, and it was deemed advisable to repeat the experiments using the method of anesthesia employed by McSwiney and Wadge.

Methods. Cats were the experimental animals, and seven experiments were performed in all. Five of the animals were anesthetized by injecting about 0.25 gram of luminal sodium intravenously per kilogram of body weight, and employing ether inhalation when necessary to eliminate contractions of the skeletal muscles. Of the remaining two, one was decerebrated, and the other was prepared by severing the spinal cord at the base of the skull and pithing the brain—these operations being carried out under ether anesthesia. In addition, both vagi were isolated and severed in the neck; a tracheal cannula was inserted, and artificial respiration was instituted and continued throughout the experiment. The cardiac fibers of the left vagus were severed in the thorax to eliminate inhibitory effects on the stomach arising indirectly from inhibition of the heart. This precaution was taken inasmuch as Veach (3) has demonstrated the prompt relaxation of the musculature of the stomach in response to diminished blood supply.

Make and break shocks of an inductorium, sent through glass shielded platinum electrodes, were used to stimulate the nerves. Shocks of moderate intensity to the tongue were usually employed. They were produced, for low frequencies, by a hand operated contact key in the primary circuit, and for the higher frequencies (about forty-five interruptions per second) by the automatic electromagnetic vibrator of the inductorium. A balloon and water manometer method, similar to that previously described (1), was used to record the contractions of the stomach. As in preceding papers, the vertical distance between the two horizontal lines above the signal magnet record, in the accompanying figures, represents a change of 5 cc. in the volume of the stomach balloon.

RESULTS. The results were uniform in all of the experiments. For moderate degrees of tonus, low frequencies produced motor effects whereas

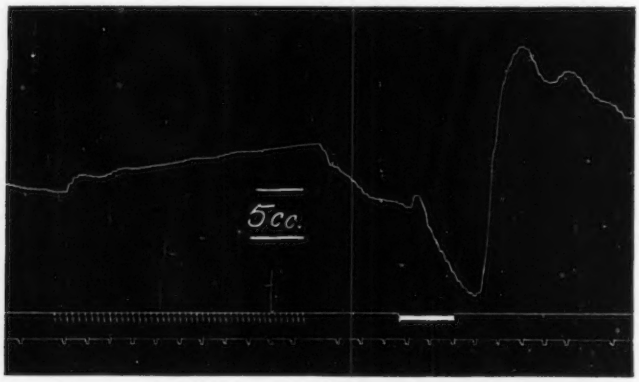

Fig. 1. Tracings from below upward: time in 5 second intervals; signal magnet record; stomach volume. Signal magnet displaced 2 mm. to left of stomach record.

high frequencies were inhibitory. The inhibition was preceded, as a rule, by an initial contraction and followed by an after-contraction. The after-contraction was quite prolonged, several minutes usually elapsing before the tonus of the stomach returned to the level preceding stimulation. Here as in the earlier experiments (1) a high degree of tonus favored the production of inhibition with both low and high frequencies. The results, therefore, were in full accord with those described in Veach's original paper.

The effect of low frequencies is shown in figure 1, A, and in figure 2. In the former, a frequency of one interruption in 1.25 seconds produced a steadily rising contraction. Obviously time was not allowed for the contraction to develop to its maximum. On cessation of stimulation, the stomach gradually relaxed to a tonus level slightly lower than that preceding stimulation. A frequency of 45 per second then produced the typical

inhibitory results, viz., an initial contraction followed by inhibition. On cessation of stimulation, the usual prolonged after-contraction occurred.

Figure 2 represents an observation from the same experiment, but made toward the end of it. Here a frequency of one in 1.5 seconds caused a gradually developing contraction, but this effect was quickly changed to inhibition when the frequency was increased to three per second. On cessation of stimulation a distinct after-contraction occurred.

As in the earlier experiments (1), it was found that the stomach showed a marked tendency to escape from inhibition. Escape usually began if stimulation was continued longer than 20 or 30 seconds. The duration of inhibition appears to depend to some extent on the condition of the animal, however, for in figure 2 there is very little tendency to escape after 47 seconds of inhibitory stimulation.

It may be added that evidence was obtained in these experiments that low intensities of stimulation tended to produce pure motor effects whereas high intensities were inhibitory, the frequency remaining constant. In

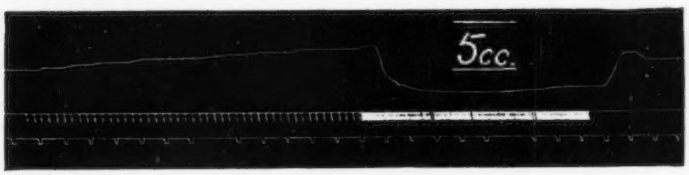

Fig. 2. Tracings from below upward: time in 5 second intervals; signal magnet record; stomach volume. Signal magnet displaced 2 mm. to left of stomach record.

one experiment, furthermore, a sufficient amount of curare was injected to paralyze the skeletal muscles, and the apparent effect was abolition of the initial contraction preceding inhibition.

Discussion. This demonstration of the relationship between vagal effects on the cat's stomach and Wedensky inhibition would call into question the validity of the conclusions of McSwiney and Wadge. Obviously if the stomach is completely atonic, further inhibition cannot be produced by any frequency of stimulation. There may then be three possibilities: 1, a steadily developing motor effect, provided that the frequency of stimulation is sufficiently low; 2, an initial contraction effect followed by more or less complete relaxation during stimulation and after-contraction on cessation of stimulation, or 3, an initial contraction effect followed by escape from inhibition during stimulation, and finally an after-contraction, depending in extent on the degree of completeness of the escape. It appears that McSwiney and Wadge obtained the first effect in their figure 1 with a frequency of 6 per second, and the third in their figure 1, B, with a frequency of 51 per second. In the latter, escape from inhibi-

tion was apparently incomplete, after-contraction occurring on cessation of stimulation.

Observations show that the assumption of McSwiney and Wadge, that the effect of the vagus on the stomach depends only on the degree of tonus preceding stimulation, cannot hold. The reaction produced is frequently diphasic, inhibition usually being preceded by an initial contraction. McSwiney and Wadge recognize the occurrence of the initial contraction, but they give it no significance. On the basis of their assumption, only one of two effects would be expected—either pure inhibition with a relatively high gastric tonus or pure contraction with a low tonus. More decisive evidence against their view is afforded by the fact that for a given degree of tonus either a motor effect or a typical inhibitory reaction is produced depending on the frequency of stimulation. Such reactions are illustrated by figure 1. Here in figure 1, A, a low frequency produces a steadily developing motor effect with relaxation on cessation of stimulation to a tonus level slightly lower than that preceding stimulation. On the theory of McSwiney and Wadge, stimulation of the vagus regardless of frequency or intensity would now produce a more marked pure motor effect than that in figure 1, A. With a higher frequency, however, the contrary is found to be true, as shown in figure 1, B. The typical inhibitory reaction occurred, viz., a brief initial contraction followed by pronounced inhibition during stimulation, and on cessation of stimulation, a prolonged after-contraction. Finally it suffices only to increase the frequency of stimulation to change a motor reaction into inhibition, as illustrated by figure 2 of the present article and figure 5 of a preceding article (3).

Inasmuch as the term, Wedensky inhibition, is not especially descriptive, it might be well to replace it by the phrase, inhibition through fatigue. It is understood that this fatigue involves only a conducting link over which the propagated disturbances pass to the effector, and this link is probably in or near the neural region of Langley. Incidentally Wedensky can hardly be considered as the discoverer of the phenomenon in the nerve-muscle preparation. Schiff (4) probably first described it, and Kries (5) made observations on it prior to Wedensky's publications.

SUMMARY

Vagal effects on the cat's stomach with various drugs as anesthetics, including luminal, are of the nature of Wedensky inhibition.

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CHANGES IN REFLEX RESPONSE, AND ELECTRICAL EXCITATION OF PERIPHERAL MOTOR NERVES IN EXPERIMENTAL HYPO- AND HYPERTHYROIDISM[1]

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Nervous symptoms of a definite type have been recorded as developing in some experimental animals after complete removal of the thyroid gland. These, in some instances, have been limited to observations made during a relatively short period of time after the thyroidectomy. Horsley (1885) described fibrillary twitching of the muscles in a monkey one week after thyroidectomy; Schiff (1884) gives a detailed account of the nervous symptoms in the dog and cat after removal of the thyroid, and Wagner (1884) describes an increase in response to galvanic currents. But later, MacCallum (1912), Paton, Findlay, Watson (1916) and Jacobson (1923) showed that the hyperexcitability of peripheral nerves to galvanic currents and, to a lesser extent, to mechanical stimuli were a feature of parathyroid insufficiency, due to unavoidable removal of the parathyroid glands in total extirpation of the thyroid in most carnivora.

Differentiation of the functions of the thyroid and parathyroid glands has demonstrated that the convulsions, spasms, tremors of the muscles, spasticity, tonic and clonic convulsions are also directly due to removal of parathyroid tissue adherent to or imbedded in the thyroid gland.

Sandstrom (1880), Baber (1882), Gley (1896) and Hofmeister (1896) were among the earlier workers who made use of the fact that in some herbivora the two external parathyroids were so located anatomically that they may be left intact in complete extirpation of the thyroid glands, and in these animals fatal symptoms of parathyroid tetany do not occur. Later, Kunde and Carlson (1927) demonstrated by chemical analysis of the blood that young rabbits could be totally thyroidectomized, leaving the external parathyroids intact, without subsequent parathyroid insufficiency as indicated by the level of the serum calcium. From this it is evident that

[1] This work has been conducted under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

it is possible to study the functions of the nervous system of the rabbit at least over a long period of time after total removal of the thyroid glands, without introducing the complications of parathyroid deficiency.

METHOD. The literature on the technic of producing cretin and hyperthyroid rabbits has been previously cited (Kunde and Carlson, 1927; Kunde and Proud, 1929).

I. *The skin reflex:* (cutaneous maximus reflex) if one applies light tactile stimuli to the dorsum of a rabbit that is not frightened, but stands quietly and relaxed in the natural position, there occurs a vigorous pulling forward of the skin over the back and sides. This is especially marked if one applies the light tactile stimuli just over the tail or in the flank. This pulling forward of the skin is effected by the reflex contraction of a muscle (panniculus carnosus) fully described by Langworthy (1925). Bensley (1910) calls this muscle the cutaneous maximus. Langworthy gives a morphological study of this muscle, its genetic relationship to the pectoral musculature and its innervation (eighth cervical and first thoracic), known as the n. thoracalis ant. Langworthy did not touch on the reflex action of the muscle.

Stimuli calling forth a reflex response of the cutaneous maximus muscle are apparently limited to only one type of sensation arising from the skin. According to von Frey (1923) tactile stimuli are divided into movement, tickle, vibration and touch. Of these, touch only is effective in initiating this skin reflex. Pain induced by pinching the skin or electrical stimuli applied from an induced coil fail to elicit the response. This may be likewise said of heat and cold.

The refractory phase of the nervous mechanism involved in the reflex contraction of the cutaneous maximus muscle invites further study. If light tactile stimuli are applied over the tail at the rate of four or five per second for a short interval of time, there is rarely a demonstrable response (movement of the skin) to more than the first three or four stimuli and usually even the third response is reduced in intensity. A short rest period of several seconds followed by another stimulus again calls forth a vigorous response.

The presence or absence of the skin reflex is easily determined by observation. However, the mechanical phase of the contraction of the panniculus carnosus can readily be recorded on the smoked surface of a rapidly revolving drum (see fig. 3) by the simple device illustrated in figure 2. This reflex has been observed in the normal rabbit as early as five days after birth.

If the thyroid glands are completely removed from a young rabbit eighteen to twenty-one days old the skin reflex can still be elicited for eight to twelve weeks after the thyroidectomy. Following this it gradually diminishes and after another month completely disappears. The disap-

pearance of the reflex occurs only in absolute cretins (see fig. 1A) and is one of the most reliable signs for differentiating between partial and complete

Fig. 1. Showing cretin, *A*, and control, *B* (litter mates). These rabbits are five months old. The cretin was thyroidectomized twenty days after birth. At the time the photograph was taken there was complete loss of skin reflex in the cretin.

Fig. 2. Showing apparatus employed in making a graphic record of the skin reflex. The clips *1*, *1*, attached to the arms of the tambour catch the hair close to the skin.

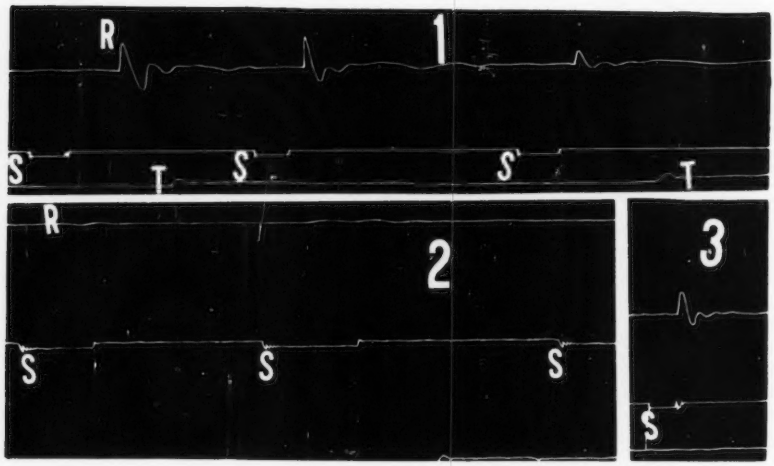

Fig. 3. Graphic presentation of the skin reflex (in rabbits) obtained by means of the apparatus shown in figure 2. *1* shows the record of a normal animal, *2* its absence in the cretin, and *3* its reappearance in the same cretin four weeks after feeding 50 to 70 mgm. desiccated thyroid daily. *S* indicates the point where the light tactile stimuli were applied over the tail. The distance between *T* and *T* is one second. *R* shows the response due to pulling forward of the skin by the contraction of the cutaneous maximus muscle

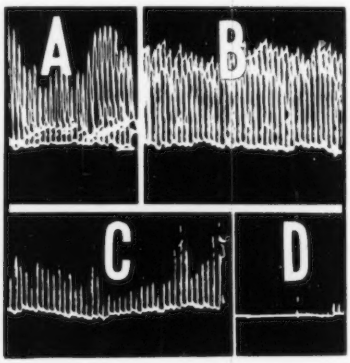

Fig. 4. Mechanogram of the knee jerk in normal and cretin rabbits. *A* = homo, and *B* = contralateral knee jerk in the normal rabbit; the force of the blow being constant and the rate of stimuli = 7 per minute, C and D show the response obtained to tapping the patellar ligament of a cretin with absence of skin reflex (the same technic was employed as in A and B). C shows the knee jerk present on the same side; D shows the absence of the contralateral knee jerk.

loss of thyroid function in the rabbit. Young rabbits with thyroids extirpated often show pronounced symptoms of thyroid deficiency, such as dwarfing in growth, potbelly, shaggy coat, anemia, etc., without developing into absolute cretins. Such rabbits do not lose the skin reflex.

After the skin reflex has completely disappeared in absolute cretins, its appearance can again be instituted by the ingestion of 70 to 100 mgm. desiccated thyroids daily. The period of time required for its reappearance is approximately two weeks after beginning the thyroid medication. If the thyroid feeding is continued and pushed to the point where severe hyperthyroidism is apparent, the reflex may persist for three or four months after the ingestion of thyroid is discontinued. Following this it again disappears. Figure 3 shows records of the contraction of the cutaneous maximus muscle in 1, the normal rabbit, 2, its disappearance in the cretin and 3, its return in the same experimental cretin after thyroid feeding has been instituted for eighteen days.

In the normal intact animal transection of the cord abolishes the reflex below the lesion. Light ether anesthesia abolishes the reflex. Section of the eighth cervical and first thoracic (thoracalis ant.) close to where they emerge from the brachial plexus causes a disappearance of the reflex on the transected side. This evidence, together with the fact that light touch initiates the reflex, proves that the afferent components of the arc are included in the cutaneous sensory fibers and the efferent arc, the eighth cervical and first thoracic nerves. The following experiments indicate that in the cretin rabbit with complete loss of skin reflex, the disturbance cannot be localized in the motor component of the reflex arc, (i.e., the n. thoracalis ant., the cutaneous maximus muscle or the motor end organs in the muscle): A cretin rabbit with loss of skin reflex may be lightly anesthetized, an incision in the dorsum of the skin made from the scapula to the iliac crest about 5 cm. lateral to the vertebral column and parallel with it, and the skin over the entire side reflected. The motor nerve to the m. cutaneous maximus can then be easily seen crossing the axilla and entering the muscle near the most anterior nipple. From this point it runs caudad parallel to the nipple line and for some distance is accompanied by a large vein which can be readily seen and serves as a valuable guide for the localization of the nerve. The nerve may be sectioned near the point of entrance to the muscle and the peripheral end stimulated with light induced shocks. A vigorous contraction of the skin of that side and movement of the tail occur in spite of complete failure to elicit the skin reflex by sensory stimuli. Or one does not need to resort to anesthesia and dissection. The unanesthetized cretin with absence of skin reflex may be placed on its side and the different electrode of the wall plate placed on the outer surface of the skin over the nerve to the cutaneous maximus muscle at the point where it enters the muscle. This point is

readily located on the outer surface of the skin by the same landmarks which have already been mentioned, i.e., the most anterior nipple and the large vein which is also easily detected on the surface of the skin. The proper cathode closing stimulus calls forth a contraction of the muscle with pulling forward of the skin in the absolute cretin the same as occurs in the normal rabbit. In this last test the usual precautions are observed in removing the hair over the areas which come in contact with the electrodes, and the different electrode is placed on the opposite side to the indifferent one. These findings indicate that the involvement lies either in the receptors in the skin, the afferent fibers of the reflex arc, or the central association mechanism. Histological examination of the skin reveals an abundant myxedematous infiltrate which may cause degenerative changes in the sense receptors of the skin thereby preventing proper excitation of the sensory nerve fiber; or the myxedematous infiltrate in and around the sensory nerves may cause functional deficiencies in these fibers; or the correlation neurons in the central mechanism may be involved.

This skin reflex should not be confused with the group of reflexes classified by some textbooks as "skin reflexes" and include the epigastric, cremasteric and gluteal reflexes. It is probably more closely related to the fibrillary twitching seen in the skin of horses and cows following the sting of a fly.

II. *The knee jerk:* striking the ligamentum patellae of a normal rabbit not only causes extension of that leg but the contralateral leg also extends (see fig. 4, A and B). We are unable to find previous mention of this contralateral knee jerk in normal rabbits and at this time have no experimental evidence to explain this physiological state, which is contrary to that which ordinarily occurs in such animals as the dog where walking is the method of locomotion.[2] In normal rabbits the contralateral knee jerk seems to be in keeping with their hopping method of forward progression. Hopping involves extension of both hind legs at the same time, and in these animals the knee jerk center is evidently normally adjusted to send motor impulses to both hind legs in response to a given stimulus. In the absolute cretin rabbit (see fig. 4, C and D) 4 to 5 months after thyroidectomy, this mechanism is impaired so that either the contralateral knee jerk does not occur at all, or it is markedly diminished. Feeding desiccated thyroids causes a return of the contralateral knee jerk. Its disappearance after discontinuance of the thyroid ingestion has not been observed. In both cretin and normal rabbit converted into a state of severe hyperthyroidism by feeding thyroid substances, the knee jerk is accompanied by foot clonus. This seems unusually interesting inasmuch as the same phenomenon has been observed in semi-cretins 12 to 14 months

[2] Orbeli states (personal communication) that he has observed the contralateral reflex in the dog after operations on certain levels of the cord.

after incomplete removal of the thyroids. The apparatus employed in obtaining the graphic record of the knee jerk has been fully described by Johnson (1927).

TABLE 1

Summary of variations in electrical excitability of peripheral motor nerves (n. thoracalis ant.) in normal, hypo- and hyperthyroid rabbits. Galvanic current from a clinical wall plate was employed

Intact unanesthetized rabbits are employed. The electrical stimuli are applied to the n. thoracalis ant., which is the motor nerve to the m. cutaneous maximus. The results are expressed in milliamperes (M.A.). The greatest number of M.A required to produce a contraction of the cutaneous maximus muscle in the 10 trials are tabulated under *high*. The least under *low*, and the average under *av*.

NUMBER OF RABBIT	C. C. C.			A. O. C.			A. C. C.		
	High	Low	Average	High	Low	Average	High	Low	Average
				Normal rabbits					
	M.A.	*M.A.*	*M.A.*	*M.A.*	*M.A.*	*M.A.*	*M.A.*	*M.A.*	*M.A.*
I	4.1	0.7	2.23	14.5	1.9	7.86	7.0	0.4	3.11
II	3.2	0.8	1.76	23.0	1.3	8.27	13.0	1.9	5.75
III	5.0	2.0	3.22	17.0	8.5	12.12	10.0	4.4	7.43
IV	3.6	1.5	2.71	20.0	7.2	13.7	9.9	3.6	5.56
V	5.0	1.0	2.61	11.5	8.0	8.65	14.5	3.4	8.49
VI	2.6	1.0	1.43	4.5	2.0	3.06	3.2	2.2	2.60
VII	2.2	0.6	1.43	4.0	0.4	2.55	5.6	0.8	3.21
				Cretin rabbits					
C. I	1.6	0.8	1.07	5.6	2.8	3.77	3.8	1.4	2.44
C. II	7.6	0.8	3.63	24.0	12.0	18.7	10.0	2.2	5.88
C. III	4.0	1.8	2.35	23.0	6.4	10.75	7.8	1.0	4.57
C. IV	7.2	0.3	3.11	23.0	3.0	9.0	13.5	0.7	7.65
C. V	3.0	1.6	1.97	10.0	2.8	6.4	7.2	1.2	5.38
C. VI	2.4	0.8	1.75	4.0	1.8	3.7	7.2	1.2	5.38
				Hyperthyroid rabbits					
H. I	2.5	1.1	1.5	5.0	1.8	3.0	3.1	2.0	2.5
H. II	2.2	0.8	1.5	11.0	10.8	10.5	4.0	2.4	3.0
H. III	2.0	2.0	2.0	11.5	8.0	9.0	7.0	7.2	7.0
H. IV	1.0	1.0	1.0	12.2	11.0	11.5	1.8	1.8	1.8
H. V	6.4	2.2	4.0	22.0	8.0	13.0	13.0	4.2	8.8

III. *Electrical excitability of peripheral motor nerves.* Since it has been demonstrated by chemical analysis of the serum calcium that these cretins suffer no parathyroid deficiency, it seems advisable to test the electrical excitability of the peripheral motor nerves in these animals, which give every known evidence of complete thyroid deficiency but manifest no

symptoms of parathyroid insufficiency. The point of departure of the motor nerve (n. thoracalis ant.) to the cutaneous maximus muscle has already been described. Its ease of localization and accessibility, together with the obvious pulling forward of the skin produced by each twitch of the muscle makes this nerve and muscle a valuable asset for physiological investigations, involving methods directed towards testing electrical excitability of peripheral motor nerves. Table 1 contains a summary of the results of our investigations of the electrical excitability of motor nerves in normal, absolute cretin and experimentally induced hyperthyroid rabbits. In these experiments an ordinary clinical wall plate was employed and the electrical excitability was tested ten different times in each animal. In order to conserve space, only the greatest, least, and average number of milliamperes required to produce the C.C.C., A.C.C. and A.O.C. response to galvanic current for each animal is tabulated. According to this method neither hypo- nor hyperthyroidism experimentally induced causes variations in the electrical excitability of the peripheral motor nerves (n. cutaneous maximus) which fall beyond the range of variations found in the normal rabbit.

SUMMARY

The skin reflex (cutaneous maximus) is present in all normal rabbits. This reflex is elicited by applying light tactile stimuli to the skin of the back of the rabbit. A response to the stimulus is manifest by a vigorous pulling forward of the skin due to the reflex contraction of the m. cutaneous maximus.

The skin reflex completely disappears in absolute cretins, experimentally induced, approximately four months after extirpation of the thyroid glands. This reflex persists in semi-cretins and serves as a reliable guide in differentiating between partial and complete thyroid insufficiency.

Ingestion of thyroid substance to a complete cretin causes a return of the skin reflex after a latent period of about 20 days. After discontinuing the thyroid substance the reflex persists for 2 to 4 months.

The contralateral knee jerk disappears in untreated cretin rabbits with loss of skin reflex. In experimental hyperthyroidism in rabbits the knee jerk may be accompanied by foot clonus.

Electrical excitability of peripheral motor nerves (n. thoracalis ant.) is not appreciably altered by experimental hypo- or hyperthyroidism in rabbits.

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THE RATE OF PASSAGE OF INERT MATERIALS THROUGH THE DIGESTIVE TRACT

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In connection with some experimentation in 1915, the writer ingested about 300 grams of glass beads in place of a meal. As a consequence, a serious condition of constipation developed which again cleared up with the passing of these beads. As the opposite effect was expected, the writer was more impressed than otherwise that glass beads pass through the digestive tract much slower than normal food residues. This impression was put to a further experimental test immediately after the appearance of the paper of Alvarez and Freedlander (1924), who used glass beads as a test material to determine the rate of progress of food residues through the bowel. Their technique was followed, but for comparison with the passage of colored glass beads, determinations were also made with cellulose (in the form of knots made of colored cotton thread or string), various seeds, gravel, steel ball-bearings, and pieces of silver. The results of five weeks of daily tests upon the writer were reported in a privately published paper (1924), which was distributed primarily among American gastro-enterologists. As this paper is now out of print, the table summarizing the data then obtained is herewith reproduced as table 1. As may be seen, the previous belief that glass beads pass through the digestive tract slower than material which is more like ordinary food residues was supported and, moreover, the data indicated that the rate of passage of the various substances then tried was more or less proportional to their specific gravity, heavier materials passing slower than light material. However, since 1924 and down to recent date (1928a, 1928b), Alvarez has repeatedly reiterated the findings obtained with glass beads as evidence of the normal intestinal rate. In view of this situation, it seemed desirable to determine whether the results obtained in personal experimentation were not more generally applicable. Accordingly, the present study of the rate of passage of a number of inert materials through the digestive tract of various laboratory animals, besides the author, was undertaken.

Observations were made on 16 rabbits, 7 guinea pigs, 50 rats, 8 mice, 4 dogs, 2 cats, 1 monkey, 3 pigeons, 1 chicken, and the writer.

The chief test materials were colored glass beads, colored glass balls, knots made

of colored cotton thread or string, and pieces of rubber, aluminum, steel, silver, and gold. The knots were soaked in soft paraffin so as to prevent their disintegration and the leaching out of their colors, in the digestive tract, as much as possible. The colored glass balls were made by fusing colored glass beads on a piece of charcoal or on a soft asbestos block. Silver and gold balls were made in a similar way from pure silver wire and 24-karat sheet gold or gold wire. Some of these metal balls were then flattened into discs, by hammering, and some were further differentiated by marking them with lines and spots, using a small chisel and center-punch, respectively. Steel was in the form of ball-bearings of various sizes. Aluminum was shaped into balls and short round-ended rods. Rubber test pieces were cut or punched mainly from black, red, and white rubber tubing.

Generally the smallest practical sizes and amounts of test material compatible with obtaining clear results were used. Tests were usually made daily for a week or more on each animal. Glass beads of the same size as those employed by Alvarez

TABLE 1

Reproduced from table 8, summary of results (Hoelzel, 1924), showing that materials passed through the digestive tract at rates proportional to their specific gravity

TYPE OF MATERIAL	NUMBER OF DETERMINATIONS	TOTAL NUMBER OF PIECES OF EACH TEST MATERIAL	PER CENT RECOVERED	AVERAGE RATE OF PASSAGE	APPROXIMATE SPECIFIC GRAVITY
				hours	
Gas (CO_2)				2 to 6*	
Food				25*	0.9 to 1.6
Tomato seeds	6	75	100	25.44	
Knots (cellulose)	40	930	99.37	26.76	1.45
Millet seeds	6	80	100	28.96	
Grape seeds	8	160	100	29.89	
Glass beads	30	800	99.63	40.15	2.60†
Gravel	3	20	100	52.31	
Steel ball-bearings	3	20	100	79.98	7.70
Silver (bent wire)	4	35	100	81.88	10.53

* The rate of passage of gas was estimated, as explained in the text. The rate of passage of food (residues) was judged by the rate of passage of various undigested food fragments and fibrous residues that were recognized in sieving the feces.

† The differently colored glass beads varied somewhat in specific gravity.

and Freedlander—about 2 mm. in their largest diameter—were used in most of the determinations made on the rabbits, dogs, cats, monkey, pigeons, chicken, and man. Other test materials used on them were mainly of comparable size. Most frequently, an equal number of pieces of two or more kinds of test material, mixed and in a capsule, was given at each trial. The capsules, in the work on rabbits for example, were introduced directly into the esophagus of the animals with the aid of a metal tube fitted with a plunger. The tube used on mature rabbits was a $\frac{1}{4}$ inch (about 6.3 mm.) cork borer that had its cutting edge blunted. The end of the tube was also slightly spread or tapered so as to hold a no. 4 (Lilly) gelatin capsule in such a way that only the larger round end would remain protruding, while the rest of the capsule was wedged snugly in the end of the tube. The end of the tube with the capsule in place

was dipped in a little corn oil before being passed into the esophagus of the rabbits. When in the esophagus, pressure on the plunger released the capsule. In a similar way, no. 5 (Lilly) capsules were given to young rabbits (or older ones) that could not easily swallow no. 4 capsules.

The guinea pigs and rats were given smaller glass beads and other test material of proportionate size. These beads were only a little over 1 mm. in their largest diameter and about 450 of them would pack into the space of 1 cc. The test material was injected into the esophagus of the rats and guinea pigs by means of a directly loaded small brass tube, which was also fitted with a plunger. This tube was about 9 cm. long with an outside diameter of $\frac{3}{32}$ inch and an inside diameter of $\frac{1}{16}$ inch (about 1.6 mm.). After the tube was loaded with test material, usually mixed and with the tube not more than half filled for a single injection, the free end was temporarily sealed with a little food. The loaded tube was then passed into the esophagus and the material was injected by adjusted pressure on the plunger. Mice were given only glass, silver, and gold balls, usually about 0.6 mm. in diameter, with a similar but smaller tube.

With the exception of the dogs, cats, and some rats while with young litters, the animals were kept in cages with screen bottoms. Feces were collected either when passed, every 24 hours, or more frequently, depending upon the conditions under investigation. The amount of feces was often measured and their character noted. To recover the test material, the feces were sieved with simple open sieves in a current of water.

Fluoroscopic observations of some animals were made from time to time when opaque test materials were used. X-ray films were obtained in some instances. The x-ray interpretations were also checked by killing some animals while under fluoroscopic observation and then determining the distribution of the test material by separately sieving the contents of various segments of the digestive tract. Stereoroentgenograms were made to determine the location of metal test material in man.

Further work on man (part 1). Since the experimentation reported in 1924, the writer observed the intestinal rate on himself by taking from 5 to 100 knots (of cotton fiber) daily and noting their rate of passage during two periods, totaling over 3 years. During the more recent studies, the writer lived in the laboratory—an arrangement which also facilitated close observation of the animals. Typical results obtained on man, when other materials besides knots were taken, are indicated in figure 1. On the whole, the earlier findings summarized in table 1 were repeatedly confirmed. The use of gold (sp. gr. = 19.2) and of aluminum (sp. gr. = 2.7) besides silver and steel has further helped to emphasize that the rate of passage is proportional to the specific gravity of the test material and not due to differences in some other respects. Although gold passed slower than any other material tried, it passed as fast as knots when it was imbedded in sufficient cork so as to make the combination no heavier than knots. However, tests, in which knots and simple pieces of cork were taken for comparison, indicated that substances lighter than the usual food residues, like cork, tend to pass slower. The rate of 2 to 6 hours given for gas in table 1 was an estimate based upon the effects of some gas-forming foods, such as lactose in personal use. But the specific effect of the quantity or

bulk of the gas and the irritating effect of other incidental products, like lactic acid, were not then taken into consideration, and it has also become evident that the rate of passage of a gas, liquid, or powder is not directly comparable with the passage of discrete solid particles like knots or glass beads. An attempt was made to determine the rate of passage of material lighter than solid food residues by swallowing small balloons, 3 to 4 mm. in diameter, but all were collapsed when passed. Moreover, because of the relatively large proportion of rubber in such small balloons, they were heavier than cork. Very likely, cork passed slower than knots or rubber

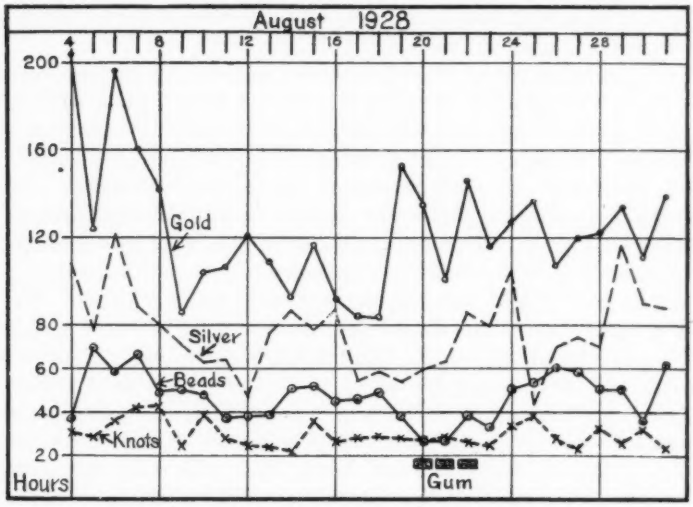


Fig. 1. Showing the rate of passage through the digestive tract of man (F.H.) when 10 knots (made of cotton thread), 10 glass beads, 10 pieces of silver, and 10 pieces of gold were taken daily. Gum is karaya gum added to the diet, as explained in the text, and causing faster passage of heavier material (glass beads).

because it would tend to float in the stomach and possibly also elsewhere. Belching is evidently facilitated by a similar tendency of gas to float or to rise. Likewise, gas in the intestines may thus accumulate at favorable sites and fail to move along as uniformly as liquid or solid matter. Such aberrant behavior of gas may be an important factor in the development of meteorism and gas pains.

A matter that was not considered in securing the earlier data is the more recent observation that test material taken when the stomach is empty and when the rest of the digestive tract is relatively empty usually passes about 12 hours faster than when taken after a meal. On one occasion,

there was a relatively thorough bowel evacuation before any food was ingested. Then, 10 knots were taken. Shortly after that, food was eaten which led to a mild further urge to defecate. Thus 1 of the 10 knots was passed 1½ hours after it was taken. This was not an instance of frank diarrhea but was the fastest rate ever noted on the writer. Rates of from 3 to 6 hours were not uncommon when there was little material in the digestive tract to block the initial peristaltic waves or rushes after eating. The fast rates which are sometimes observed when barium sulphate is taken, after abstinence from food or after purging, may be due to similar circumstances. It has therefore seemed best, when aiming to determine the rate of passage of food residues, to take test material only after a portion of the food has been eaten.

It was believed (1924) that glass beads passed through the digestive tract faster than glass balls would be likely to pass because food debris might fill the holes in the beads and thus reduce the net specific gravity of the mass. However, repeated tests in which both beads and glass balls were used showed no clear-cut difference in the rates of passage. On the other hand, small coils of iron wire (wound loosely like small springs and with the ends turned in) passed much faster than steel ball-bearings (sp. gr. practically the same as iron) and sometimes passed faster than glass. Very thin discs of gold also passed much faster than gold balls. The dispersion of the mass or the considerably increased surface area apparently helps to speed up the passage. Similar results were obtained when from 12 to 35 grams of barium sulphate (sp. gr. = 4.5) or 3 grams of ferric oxide (sp. gr. = 5.2) were taken. The bulk of these substances passed as fast as knots that were taken at the same time, but here the general intestinal rate was often slowed down. The barium seemed to slow down the passage in proportion to the increased specific gravity of the intestinal contents but the ferric oxide had an effect out of all proportion to the small amount taken. Moreover, the slowing was then a delayed effect or after-effect and was probably due to an astringent action which Bergeim (1924) referred to in suggesting the use of this oxide for intestinal studies.

The black, red, and white rubber which was used differed a little in specific gravity but tests on the writer showed no appreciable difference in the rates of passage, either among the different types of rubber or of rubber when compared with knots. However, some colored glass beads differed enough in specific gravity to show distinct differences in the rates of passage. The beads used in obtaining the data represented in figure 1 were of practically equal specific gravity.

With this preliminary, the results of the animal studies will next be presented and further results of human experimentation will be referred to after that.

Rabbits. The idea of using glass beads for determining the intestinal

rate was credited to Elliott and Barclay-Smith (1904) by Alvarez and Freedlander. The former workers studied the distribution of food along the digestive tract of rabbits by giving them differently colored glass beads daily during a short period before killing and examining them. Alvarez and Freedlander also repeated such experimentation on rabbits and claimed to have obtained results similar to those reported by Elliott and Barclay-Smith. But in view of the data obtained by the writer upon himself, it was believed (1924) that the rabbit studies with glass beads could not have revealed the normal distribution of food in the digestive tract. To test this opinion experimentally in the present study, from 8 to 40 knots or pieces of rubber were given with from 8 to 100 glass beads or glass balls on 83 occasions to 16 different rabbits. Because of practical and natural difficulties, 100 per cent of *both* types of the contrasting materials were recovered in only 22 of these tests. Knots or rubber passed faster than glass 11 times, glass passed faster 10 times, and 1 time no difference in rates was noted. In these tests therefore glass was not clearly retarded in passing through the digestive tract of rabbits. An explanation of this unexpected result was obtained when killed animals were examined. It was then noted that in those cases where glass passed faster, it left the stomach sooner than knots or rubber. This evidently occurred because glass adhered less to the fibrous mass of food in the stomach and consequently passed out through the pylorus easier. Clear evidence of a similar differential retardation in the cecum was not obtained. A small portion of glass was sometimes found in the appendix a month after it was given but knots and rubber disintegrated beyond recognition if not passed within 6 days. This was one source of the frequent failure to recover 100 per cent of such test material.

The failure to find clear evidence in rabbits that glass tends to pass through the digestive tract slower than knots or rubber was largely compensated by the finding of the greatest stasis of gold observed in any species studied. Steel and silver were uniformly slower in passing than aluminum, glass, rubber, and knots, but passed distinctly faster than gold. There were great individual variations. For instance, the first two rabbits studied were both females but one had a somewhat slower rate for light material (aluminum, glass, rubber, and knots) than the other. When given 5 pieces of gold, the faster rabbit passed all in 5 days and passed all gold in 3 days on two subsequent occasions, but the slower rabbit passed only 1 out of 5 pieces of gold 2 days after it was given and no further gold before the animal was killed, 22 days later. Both of these rabbits were given larger amounts of gold, of different pattern, a few days before they were killed. It was then discovered that *the gold given last was retained chiefly in the duodenum of both animals.* Of the first 5 pieces of gold given to the slower rabbit, 1 piece was still in the stomach, 1 in the duodenum, and 2 in the

appendix among other test material. The faster rabbit had less gastric stasis of other material and nothing in the appendix. Fluoroscopic observation proved that the killing of the animals had not caused any noteworthy change in the distribution of the metal throughout the digestive tract.

Why should the duodenum of rabbits be a common site of stasis of heavy material like gold, as the foregoing and subsequent observations indicated? Three reasons suggest themselves. First, the intestines of rabbits are poorly supplied with muscle tissue and hence heavy materials are not passed along as well as in the more powerful intestines of other mammals; second, the sedimentation of heavy material is favored more in the duodenum than elsewhere in the digestive tract of rabbits because this segment generally contains a more nearly liquid media (chyle), and third, the duodenum of rabbits is a long loop ending in a sharp duodenojejunal flexure which seems to serve partly as a mechanical barrier to the free passage of solid material. The duodenum of rabbits therefore may be regarded as functioning more like the stomach in many other animals. No other species of mammal studied was observed to have a comparable stasis of gold in the duodenum.

The question was next raised whether complete duodenal obstruction could be produced in rabbits by giving sufficient gold. Briefly stated, after being given liberal amounts of gold during periods of varying length, 5 rabbits died and 3 others were allowed to recover after death seemed certain if more gold were given. However, although as much as 65 grams, or 56 per cent of all the gold in the digestive tract, was found in the duodenum of one rabbit, it seems that complete obstruction could not be produced at this site, at least not when round pieces of metal were used. The degree of obstruction was determined by giving light test material regularly and noting how much passed and when. In one case where small rectangular blocks of gold were employed, complete duodenal obstruction apparently occurred. But round pieces of gold formed a loose mass through which food, digestive juices, knots, rubber, glass, and even steel and silver passed or wormed their way. Obstruction was also prevented by the dilatation of the duodenum. The presence of much heavy material in the duodenum however usually stimulated violent peristalsis. This was indicated by the development of some degree of diarrhea in all instances and often mass movements of all the metal down to the cecum or colon would occur. Such a mass movement very likely explains why one animal died suddenly with an intussusception of the colon, near the hepatic flexure, and with nearly all of the gold in the cecum. Two rabbits died with complete obstruction of the colon at the hepatic flexure. Here obstruction tends to become complete because the partly dehydrated food residues are likely to fill up the interstices between the pieces of metal without passing by as in the duode-

num. The two other animals probably died mainly of starvtion. This was due to eating insufficient food after partial obstruction occurred and because of the development of intestinal inflammation, melena and/or lesions which were presumably caused by mechanical irritation together with some obstruction. The distribution of the metal in one of the rabbits that died with the colon dilated and obstructed was as follows: stomach, 17.9 grams; duodenum, 32.1 grams; jejunum and ileum, 0.2 gram; cecum, 6.3 grams; appendix (all in apical end), 12.2 grams; dilated and obstructed part of colon, 18.3 grams; rest of colon, 11.3 grams; total 98.3 grams. This may seem like an enormous quantity of metal, but because of the high specific gravity of gold, all this metal could be packed loosely into the space of 11.5 cc. It is therefore not the bulk of the material but its high specific gravity that makes a liberal amount of gold in the digestive tract a fatal burden to most rabbits.

Another question which the writer tried to settle by experimentation with rabbits was how a marked stasis of glass beads throughout the digestive tract, such as the example which Elliott and Barclay-Smith gave in detail, might occur. In 154 tests, 100 per cent of the glass beads, glass balls, knots, or pieces of rubber, which were given either alone or with heavier test material, were recovered. Passage of 100 per cent occurred as follows: 3 times within 10 hours, 7 additional times within 15 hours, 27 times within 24 hours, 61 times within 48 hours, 30 times within 72 hours, and the balance of 26 additional times within 7 days. The slower passages usually occurred when the animals were partly obstructed and somewhat faster rates might have been noted if the feces had been collected at closer intervals. There were no occasions when a substantial percentage of glass beads or other light material remained in the digestive tract of a healthy, unobstructed rabbit more than two days. It was therefore believed that a general stasis of beads might have been due to a failure of the experimental animals of the previous observers to eat normal amounts of food. This might have happened if the throats of the animals had been injured or considerably irritated by the method of giving the beads. The effect of decreasing the food allowed by 50 per cent of the amount previously eaten spontaneously was determined on one animal for a few days before it was killed. The result was that the rate of passage of test material was slowed down considerably but there was no marked general stasis. Another rabbit was therefore given only water and test material for 4 days before it was killed. In this case, there was a stasis of test material similar to that in the example given by Elliott and Barclay-Smith, excepting that the appendix only contained 1 out of 30 pieces of rubber given 3 days before starvation was instituted. In spite of starvation, a little test material given on the third day was passed on the fourth or last day of starvation. The passage of some feces and test material is therefore no proof that a rab-

bit has eaten a normal amount of food. In this study, the quantity of feces passed by the rabbits was always measured by collecting them in a graduate. Marked variations in the quantity of feces and of test material passed were thus observed to be due either to changes in the food intake or more or less obstruction.

The rabbits in this study were under continuous observation for periods ranging from 10 to 137 days, average 51.4 days. It is possible that this more prolonged study accounts partly for results that differ from those reported by previous workers. It was repeatedly seen that data secured during the first few days frequently differed from data obtained subsequently. Psychic disturbances of the animals during the initial days may account for the atypical results, but this was not always obvious.

Guinea pigs. Of the 7 guinea pigs observed, 2 females and 2 males were given a variety of test material during 20, 28, 22, and 106 days, respectively. The 3 others (males) were given liberal amounts of gold only and were observed from 30 to 34 days. The guinea pigs, as well as all other species studied with the exception of the rabbits, passed knots or pieces of rubber faster than glass beads or glass balls. The relative difference in the rates of passage of materials differing in specific gravity was greater in guinea pigs than in most other animals studied. Typical results are indicated in the data appended below. Knots and white and red rubber were disintegrated beyond recognition in 48 to 72 hours or more than twice as fast as in rabbits. In single tests on two guinea pigs, short rods of aluminum passed much faster than glass, possibly because they were somewhat irritating. The greatest stasis of gold, silver, and steel occurred in the stomach while lighter material usually remained longest in the cecum. The gastric stasis of heavy material was evidently due to 1, a poorly developed gastric musculature, and 2, the fairly constant presence of large amounts of semi-liquid contents which favored the sedimentation of heavy material and also prevented the discharge of such residuum. A specific stimulus to the evacuation of heavy material from the stomach seemed to be a large accumulation, due to repeated injections. Then mass movements would generally take place in which all of the metal might be discharged from the stomach. A transient secondary accumulation would next occur in the proximal end of the cecum. In contrast to what often happened in rabbits, heavy material did not tend to go to the apical end of the cecum. Light material was better distributed. A mass of metal in the proximal cecum sufficient to form a single large fecal pellet appeared to be a specific stimulus for a mass movement from this site. Fecal pellets containing heavy metal often contained little else and were frequently preceded and followed by numerous pellets containing no heavy material whatever. On the other hand, light test material was well distributed among the fecal pellets.

For injecting material, guinea pigs proved to be difficult subjects. Five of the 7 animals were thus injured. Ether anesthesia, to facilitate the giving of material, was found to be unsatisfactory. The two females proved to have been pregnant. Both aborted during the course of experimentation. After abortion their intestinal rates became somewhat faster. The males showed less stasis than the females, but injured males that developed abscesses showed more stasis than the pregnant females. The spontaneous rupture of abscesses was always followed by improved gastro-intestinal motility.

Examples of tests and results. Guinea pig 2, female, given 6 black-and-white knots and 8 red glass balls. Feces collected after 12 hours contain 3 knots, no glass balls; after 34 hours, 1 more knot, no glass; 47 hours, no more knots, 1 glass; 105 hours, 1 glass; 11 days, 1 glass; 13 days, 1 glass; 14 days, 2 glass; 15 days, 1 glass; 18 days, 1 final glass. The 2 missing knots were evidently disintegrated.

Guinea pig 4, male, given 8 black-and-white knots and 8 red glass balls. Passed within 10 hours, 1 knot, no glass balls; 24 hours, 2 more knots, no glass; 48 hours, no more knots, 1 glass; 58 hours, 2 glass; 7 days, 1 glass; 12 days, 1 glass; 13 days, 2 glass; 14 days, 1 final glass. The 5 missing knots were very likely disintegrated.

Guinea pig 1, female, given 10 short aluminum rods. Passed within 24 hour, 5 rods; 34 hours, 2 more rods; 48 hours, 2 rods (one distinctly corroded by acid gastric juice); 60 hours, 1 final rod.

Guinea pig 1, given 10 pieces of red rubber and 10 steel ball-bearings. Passed 5 pieces of rubber within 11 hours; no more rubber recovered later. Steel passed, 2nd day, 1 ball; 8th, 1; 10th, 2; 12th, 1; 15th, 1; 16th, 2; 17th, 1; 22nd day, 1 final ball-bearing. Some steel considerably corroded by acid gastric juice.

Guinea pig 1, 15 out of 16 gold balls, given 21 days before killing, still in stomach; other gold ball in cecum.

Guinea pig 2, 6 out of 7 gold balls, given 17 days before killing, still in stomach; other ball was passed on 12th day.

Dogs. Dogs passed light material faster than heavy material but the difference in the rates of passage was not great. In fact, knots or rubber invariably passed faster than glass in only one dog, while it was being fed a semi-liquid experimental diet. Steel and silver however passed about 24 hours slower, even with a regular diet of meat and bread. Gold was not given to any dogs as they were not kept confined continually. The heavier materials, including the glass in the animal on the semi-liquid diet, passed mainly with residues accumulating during the interdigestive periods. That is, these dogs were generally fed only once daily and the residues from the meals were separated from one another by large amounts of hair, sawdust, etc., which were swallowed between meals. These animals were used chiefly in the hope of finding correlations between the intestinal rates, as determined by the writer, and the gastric secretory and motor activity (balloon method) which were primarily being observed by other laboratory workers. But it is obvious that with the swallowing of so much extraneous material, variations in the intestinal rates or in other gastro-intestinal

functions meant little. Moreover, 3 out of the 4 animals were found to resort to coprophagy and the other was suspected because some light material failed to pass in uniform sequence. In addition to this, 3 out of the 4 dogs harbored worms (noted in sieving the feces). Olsen (1928) also found considerable hair in the feces of dogs even after wire screen muzzles were attached. It was intended to make further studies on dogs later with better conditions but in the meantime Loo, Chang and Lim (1928) reported their observation that pieces of silver passed about 24 hours slower in dogs than glass beads and this confirmation of the general findings in this study was regarded as sufficient for the present purpose. Dogs evidently pass heavy material easier than rabbits, guinea pigs or man because of having a shorter and more powerful digestive tract.

Examples of tests. Dog 1, female, diet of yeast and acidophilus milk, given 20 yellow-and-white knots and 20 yellow glass beads. Passed within 6 hours, 19 knots, no beads; 14 hours later, 1 final knot, 15 beads; remaining 5 beads passed during next 31 hours.

Dog 1, diet of meat and bread, given 20 red-and-yellow knots, 20 white glass balls, 5 pieces of aluminum, and 5 steel ball-bearings. Passed after 23 hours, 14 knots, 9 glass, 3 aluminum, no steel; 20 hours later, 6 remaining knots, 10 glass, 2 remaining aluminum, no steel; after 32 more hours, all steel; (1 glass ball missing).

Cats. Both cats observed were tame males. The test material was easily given in no. 4 (Lilly) capsules, which were discharged into the back of the mouth by means of the tube used for introducing such capsules into the esophagus of rabbits. The first cat studied (7 days) often passed all gold, silver, steel, and glass within 10 hours, while knots and rubber sometimes passed a little slower. It was at first thought that posture in this cat (frequent turning over and other contortions) might account for a speeding up in the passage of heavy material. Presumably, suitable posture, conjointly with gravity, would lead to a faster passage of the heavier material. However, observations on a second cat (9 days) made it evident that the first cat had an irritable digestive tract and suffered from diarrhea. It defecated 2 or 3 times as frequently as the second cat. Cat 2 showed a fairly uniform gradation, according to specific gravity, in the passage of the different test materials.

Examples of tests on cat 2. Given 12 pieces of black rubber and 12 red glass balls. Passed after 11 hours, 9 rubber, 2 glass; 8 hours later, 3 remaining rubber, 5 glass; during next 16 hours, 5 remaining glass.

Given 12 red glass balls and 12 pieces of gold. Passed after 24 hours, 11 glass, no gold; 4 hours later, 1 remaining glass, 6 gold; after 22 hours more, 2 gold; 23 hours later, 2 gold; next 24 hours, 1 gold; last piece of gold found in cage at close of experiment 3 days later, obviously passed earlier.

Monkey. In view of the fact that data on man other than that obtained on the writer was not secured in this study, thanks are due to Professor

Tatum for the opportunity and to Doctor Seevers for assistance in securing data on a young male Macacus rhesus. Test materials were given in no. 5 (Lilly) capsules which were discharged into the pharynx with the aid

TABLE 2

Rate of passage of various test materials through the digestive tract of a young male Macacus rhesus, weighing about 3 kgm.

DATE (1928)	GIVEN	PER CENT RECOVERED	AVERAGE RATE OF PASSAGE	REMARKS
			hours	
July 2	8 red-and-white knots	75	19.1	Apparently capsule broke in mouth and missing material was not swallowed
	8 red glass beads	88	18.6	
July 3	10 red-and-black knots	100	21.3	
	10 silver balls	100	47.3	
July 4	10 white knots	100	17.6	
	10 gold balls	100	51.9	
July 5	10 round black rubber	100	20.5	
	10 yellow glass beads	100	28.5	
July 6	10 black-and-white knots	100	17.0	Missing bead perhaps lost in sieving feces
	10 blue glass beads	90	29.8	
July 7	6 black rubber	100	18.1	
	6 blue glass balls	100	17.8	
	6 silver balls	100	33.2	
	6 gold balls	100	39.1	
July 8	10 red knots	100	17.6	
	10 red glass balls	100	26.3	
July 9	10 black knots	100	21.6	
	10 green glass beads	100	33.7	
July 10	15 red rubber	100	14.8	"Rat size" material given. Capsule was probably regurgitated and broken in mouth. Rubber swallowed—recovered in feces. Other material found free in cage
	10 blue glass beads	95	—	
	10 silver balls	100	—	
	20 gold balls	100	—	
July 11	11 silver balls	100	94.7	"Regular size" balls—same as those given on 3rd, 4th, and 7th
	11 gold balls	100	103.8	

TABLE 2—*Concluded*

DATE (1928)	GIVEN	PER CENT RECOVERED	AVERAGE RATE OF PASSAGE	REMARKS
			hours	
July 12	12 red knots	100	23.0	"Rat size" material—2 capsules full
	10 black knots	100	23.0	
	12 red rubber	100	23.0	
	15 black rubber	100	23.4	
	15 blue glass beads	100	23.0	
	25 red glass beads	100	28.1	
	15 silver balls	100	65.0	
	15 gold balls	100	68.8	
Rate of passage of all—				
Knots			20.0	The diet during this period consisted almost exclusively of fruit, vegetables, grains, and sunflower seed
Rubber			20.0	
Glass			25.7	
Silver			60.1	
Gold			65.9	

of the tube used for giving such capsules to small rabbits. The monkey's cage was visited about every 2 hours between 8:00 a.m. and 10:00 p.m. and feces were collected when present. It defecated little or not at all during the nights. The results, which are given in some detail in table 2, show a gradation in the rates of passage of various materials somewhat similar to, but faster than, the rates usually obtained on man. A curious circumstance is the relatively slight difference between the rates of passage of silver and gold. Still more remarkable is the considerable variation in the rates of passage of the heavy metals given on different days, independent of a corresponding variation in the passage of light materials. The gold and silver was evidently retarded chiefly in passing through some specific segment of the digestive tract and this raised the question whether the variations in the rate of passage of the heavy metals reflected a periodic change in local motility at the site of stasis. The monkey did not seem to be a suitable subject for investigating this possibility, as it was difficult to evaluate the rôle of psychic factors in modifying the intestinal activity in such an animal. Hence, further work was done on rabbits, rats, and man in the hope of shedding light on this question. Rabbits, however, proved to be unsatisfactory for this study because of a variable degree of stasis of heavy material in several parts of the digestive tract and also because of the tendency to develop obstruction even when only small amounts of gold were given daily.

Albino rats. Most of the tests on rats were made with glass and metal but some tests were also made with knots and rubber. These showed that knots were sometimes retarded more than glass or heavy metal in

passing the pylorus. Similarly, coarse food like carrots sometimes left the stomach slower than heavy metal. This may have been due to a sieve-like function of the pyloric end of the stomach, which operated more effectively in some rats than in others. But it is also possible that a mat-

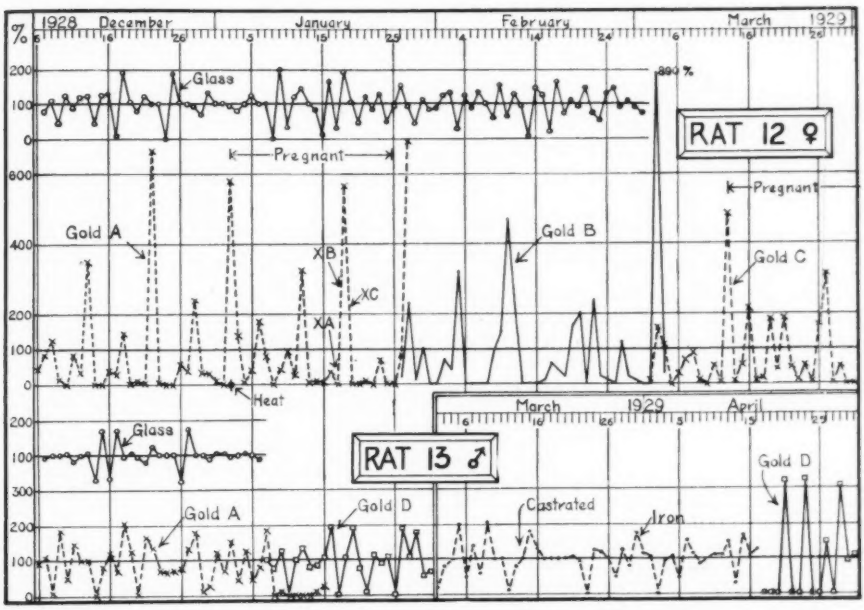


Fig. 2. Showing that the passage of a heavy material like gold tends to vary quite independently of the passage of a lighter material like glass (in rats). The curves for glass and metal show the per cent of the number of pieces of test material given daily that were passed each day. Twenty colored glass beads or glass balls were given daily to each rat during the periods indicated. Gold A, passage when 20 gold balls were given daily. Gold B, 10 double gold balls (two single gold balls, like gold A, fused together) given daily. Gold C, 40 gold balls (same type as gold A) given daily. Gold D, 20 small rectangular blocks of gold given daily. Iron = short rods of iron wire. Smear tests showed that rat 12 was in heat on the day indicated for the beginning of the first pregnancy while such tests did not show evidence of heat during the remainder of the time (3 days) that the male (rat 7) was left with her. The beginning of the second pregnancy was determined by allowing a similar period for gestation (22½ days). *XA*, *XB*, and *XC* indicate when the x-ray pictures in figure 3 were taken.

ting of more or less swallowed hair served as a screen at the pylorus. Pieces of rubber passed the pylorus easier than knots and quite regularly passed through the entire digestive tract faster than heavier materials.

The most striking phenomenon observed in rats was a periodic change in the rate of passage of heavy material which was largely, but not entirely, independent of any corresponding change in the passage of light material. This was apparently similar to the periodic variation in the passage of heavy metal first seen in the monkey. It was well demonstrated in the rats when an equal number of gold balls and glass beads or glass balls were

A B C

Fig. 3. Roentgenograms of rat 12 showing cycle of periodic intestinal activity during period when 20 gold balls and 20 glass beads or glass balls were given daily. A, taken at *XA*, figure 2, indicates relative gastro-intestinal quiesence with accumulation of 124 gold balls in cecum. Ten gold balls, given 4 hours earlier with 10 glass beads, are still in the stomach with some of the beads (3 visible). B, taken following day, at *XB*, figure 2, shows heightened gastro-intestinal activity. Fifty-six gold balls were passed and 30 were given since roentgenogram A was taken. The 10 gold balls in the stomach were introduced 5 minutes before this film was made and after a previous film had been made for comparison. Ten gold balls, given $4\frac{1}{2}$ hours earlier with 10 black glass balls, are evidently all in the terminal ileum, along side of cecum. Five of the 10 black glass balls passed 20 minutes after this film was made, along with the gold pellet shown in the rectum and containing 41 gold balls. Forty-six gold balls are left in the cecum and 1 (isolated) ball probably in the colon. C, taken next day, at *XC*, figure 2, indicates subsequent decrease in gastro-intestinal activity. Only 16 more gold balls passed after the fecal pellet with 41 gold balls, in B, passed. Then stasis of gold began again and continued more or less until after the animal littered (fig. 2). Seven out of 10 gold balls, given $4\frac{3}{4}$ hours earlier with 10 white glass beads, are still in the stomach. The other 3 are apparently in the small intestine. Of the 10 white glass beads, 3 are in the rectum and were passed 15 minutes later; 4 other white beads are also easily visible higher up in the intestine. Note that the beads advanced much farther than the heavier gold that was given with them.

given daily (fig. 2). The periodic change in the passage of the heavy metal reflected a periodic variation in the stasis of such metal in the cecum, but the activity of the rest of the digestive tract was also often involved in these periodicities (fig. 3). Great differences in the types of this periodic activity were manifested by the various individuals but, in general, the females showed more clear-cut periodicities than the males. The curves in figure 2 represent extreme types. Usually the curves were more irregular.

Inasmuch as the females usually showed more marked periodicities than the males, it was at first thought that their variations in the passage of heavy material might be related to the estrous cycle. Smear tests were accordingly made on some of the rats by Miss Esther DaCosta and some were observed in rotary activity cages while the intestinal activity was simultaneously being determined, but this work failed to show a uniform relation between the estrous cycle or the periodic changes in the running activity of female rats and the passage of heavy metal. That is, there have been many indications that the periods of heat and sexual or general activity may influence, or be related to, intestinal activity but so many exceptions have also been found that much further work would be necessary to determine whether such apparent relationships as have been seen were anything more than simple coincidences.

The spaying of a female rat and the castration of rat 13 (fig. 2) and of another male, by Miss DaCosta, led to the speeding up of the passage of heavy metal in these rats during a short period after operation. Then, in rat 13, greater stasis developed than in the pre-operative period and this increase was greater than what might be ascribed to aging, unless complicated by disease. In fact, disease was more definitely indicated later when this rat developed a type of stasis of gold such as was not seen in any other rat. Following the period indicated in figure 2 and beginning May 26th, 10 gold balls were given daily in place of the 20 gold blocks. Until August 12th, all gold given was never retained for more than 3 successive days. Then rat 13 retained all gold given for 38 successive days (380 gold balls). On the 39th day passage of gold began again with the passing of some bloody feces and 1 gold ball. However, bloody feces were already observed on the 27th, 32nd, and 36th days of gold stasis. The number of gold balls given was considerably increased after the 44th day as only 5 more gold balls were passed since the 39th day. The animal was sacrificed on the 54th day to determine the cause of the stasis of gold and the periodic passage of blood, which had continued; 169 gold balls were found in the stomach, but that was less than the number given during the last 36 hours. The animal's cecum, which was relatively small or contracted, was filled to overflowing with 889 gold balls; 45 gold balls were apparently held back in the terminal ileum; 2 were in the colon (total, 1105 gold balls in the digestive tract). Only the colon contained blood, which seemed to have come from a very small lesion about 2.5 cm. from the cecum. Histologic examination of this segment by Miss DaCosta indicated inflammation, and bleeding in the sub-mucosa. The lymph glands in this region were also hyperplastic. The stomach, small intestine, cecum, and the remainder of the colon appeared to be normal, grossly and microscopically. The rat had a pulmonary caseo-nodular tuberculosis which would largely account for the primary increase in the stasis of gold after castration, and

the condition in the proximal colon, developing later, may have led to a spastic local contraction or reflex inhibition which prevented the cecum from emptying. This would be similar to the gastric stasis which has often been seen in human cases with duodenal ulcer. The rat also became extremely nervous (much more easily frightened) after the 4th day of gold stasis, which seems to parallel the nervousness of many ulcer patients. As the rat passed feces daily throughout the period that all gold was retained, this shows how much more clearly a stasis is revealed by heavy test material than by light material. Increased stasis like that in rat 13 was not indicated by the other rats in the same length of time after operation but some increased stasis might have been masked by irregularities in the curves yielded by these rats both before and after operation. Growth was complete in all three rats before operation. The female showed periodic changes in the passage of metal after being spayed very much as before. This and the fact that the periodicities tend to continue throughout pregnancies (fig. 2) would seem to prove that there is no direct relation between the estrous cycle and the periodic variations in intestinal or cecal activity.

An attempt was then made to determine whether functional or structural differences in the digestive tracts of the rats might explain the considerable individual variations in the passage of heavy metal. Observations made on the tonus of various parts of the digestive tract of some rats, immediately after laparotomy, seemed to be of little value as the anesthetic, the resistance to its administration, and the operative insult very likely already affected the different animals differently. In killed rats, great variations in the position of the cecum and in its relation to the colon were noted, but more extensive work would be necessary to determine whether such variations can account for the differences in the passage of heavy material.

Another approach to the solution of the same problem was to estimate the rôle of heredity in determining the type of variation in the passage of heavy metal. Thirty of the 50 rats upon which observations were made were the inbred descendants of two females (rat 6 and rat 12), each mated with the same male (rat 7). Eight of the 30 rats descended from rat 6 and 22 from rat 12. Members of three generations were included in each series. As a result it was seen that some of the specific peculiarities in the way that rat 6 and rat 12 passed heavy metal appeared either only, or more exclusively, in their own descendants. In other words, there was definite evidence that the factors that determine how a specific rat will pass heavy test material are more or less hereditary.

Incidentally, the breeding experiments also indicated that some relationship exists between the efficiency with which female rats pass heavy test material and their fertility, the viability of their young, and the sex ratio in their litters. Thus, in two instances where three littermate sis-

ters were left to breed with their littermate brother, the females with the least stasis of heavy metal were the first to become pregnant. In one of these instances, the other two littermate sisters did not become pregnant in more than a month afterward; in the other case, both sisters (rat 23 and rat 22) became pregnant within 10 days after the first sister (rat 24). Rat 24 had a litter consisting of 5 males and 6 females and all survived until after weaning. Rat 23 (second pregnant) had a litter with 3 males and 5 females (a smaller litter and smaller proportion of males) and all died before weaning. Rat 22 (last pregnant) had a litter with 3 males and 8 females of which 1 male and 4 females died before weaning. Among these three sisters, rat 23 had the greatest stasis of heavy metal and greatest mortality among her young while rat 22 was the smallest animal and had the lowest sex ratio in her litter. In 3 subsequent pregnancies, rat 23 also had smaller litters than rat 24, while rat 22 had a still lower sex ratio among her young in her second litter, and her third litter was smaller. In another breeding litter with 4 females, the one with the greatest periodic stasis of heavy metal was the last to become pregnant. This, although she was the largest animal of the 4. Then she apparently developed a pseudopregnancy first. Later she underwent a regular pregnancy and had a litter of 5 females—the largest exclusively female litter among 17 litters in her strain (the family of rat 6). Again, rat 6 and her inbred descendants were characterized by having more masked stasis of heavy material and by being less fertile than rat 12 and her descendants. In the family of rat 6, there were 7 breeding females that had, altogether, 17 litters with from 1 to 6 young per litter (average only 2.95 per litter). On the other hand, only 4 females in the family of rat 12 were used for breeding, but, in a similar period, they had 14 litters with from 4 to 13 young per litter (average 8.64 per litter). This difference was present in spite of the fact that the rats in the family of rat 12 were the smaller and appeared to be the less vigorous when young. Likewise, among the entire 13 breeding females in this study of rats, rat 24 passed gold better, before her first pregnancy, than any other female. Correspondingly, she had the largest litters, with 11, 11, and 13 young, respectively (17 males and 18 females). The other 12 breeding females had, on the average, only 4.91 young per litter, with a total of 67 males and 90 females. That is, with a less efficient motor activity of the digestive tract, there was less fertility and a lower sex ratio in their litters. This may simply mean that gastro-intestinal motility and reproductive ability are likely to operate on a similar plane of fitness in the same animal. The sex ratio also may merely be a consequence of a differential fetal survival and / or the effect of metabolism on sex determination (Riddle).

Tests made throughout 35 pregnancies in the rats showed that there was an increased stasis of heavy material, provided that only moderate

amounts were given daily. When large amounts were given, a smaller proportion was usually retained toward the end of pregnancy. Perhaps pressure on the cecum, due to the growing fetuses, prevented as great an accumulation then as otherwise. Similar pressure may also explain a frequent peak excretion of metal a day or two before littering, even when small amounts were given. If a large proportion of metal was not passed shortly before littering, it was usually passed soon afterward. During lactation and probably because of the drain on the vitality of the mothers, a greater stasis of heavy material than during pregnancy often developed. The initiation of pregnancy with a peak excretion of metal, as in rat 12 (fig. 2), was not the rule. In fact, a sudden increase in stasis more frequently took place.

A complicating factor in attempting to determine the cause of the periodic changes in the passage of heavy material was the fact that some alterations in the periodicities could be produced by simply giving different amounts or different types of test material. For example, it may be seen from figure 2 that rat 12 showed, proportionately, less striking variations in the passage of gold balls when 40 were given instead of 20, and this although the giving of 20 glass beads or glass balls daily was discontinued when the gold was increased. Such results suggest that the accumulation of a certain amount of heavy material served as a direct stimulus of its passage but the condition of the animal or of the digestive tract was evidently even more important in determining the response.

Furthermore, when the same amount of heavy test material was given daily to a growing rat, increasing stasis was usually seen with increase in size and this may chiefly have been due to the fact that the uniform mass actually became proportionately smaller under the conditions. But apart from this, sharp increases in stasis were often seen about the time of maturity (60 days). As already suggested above, stasis also increased with age apart from growth and this was frequently masked by a decrease in the periodic variations in the passage of heavy metal. Such masked stasis occurred when only a part, or only a smaller part, of the accumulated material was passed at times of increased excretion. Rat 12 (fig. 2) showed such striking peaks in the curve of excretion because she often emptied her cecum completely at such times. Other rats, like most of the females in the family of rat 6, had larger accumulations in the cecum, but they passed only part and usually retained some previously given gold over a month after a change to a different pattern of gold was made. Similarly, one old male, that died apparently of tuberculosis and that showed no striking peaks of excretion, had some gold, among much other material in his dilated cecum, that was given 95 days before death. Longer stasis might have been demonstrated if gold had been given earlier. This rat also had as much silver in his dilated stomach as was given during 6 days before

death. Gastric stasis of gold or silver for more than 36 hours was not seen in healthy rats less than a year old but it was common among older rats.

The periodic peaks of excretion of heavy metal may also largely have been a consequence of the tendency of such material to pass in masses when it passed at all. Mass movements of metal were observed in rabbits and guinea pigs, as well as in rats, and to a lesser extent in mice and in man. The fecal pellet of metal shown in figure 3, B, and containing 41 gold balls and 1 red glass bead, was a relatively small pellet. Rat 6 passed one fecal pellet of metal containing 176 gold balls. Still larger pellets of metal were passed when larger test pieces of metal were given, although the number of pieces was then smaller. It is easy to see that, when so much material can be passed in a single pellet, the passage of metal is not likely to be influenced much by the amount of roughage in the diet. This was more definitely proven on man where the intake of roughage could be more rigidly controlled (fig. 4, columns).

To what extent swallowed hair may have complicated the passage of test material other than knots is a question. The amount of hair, noted in sieving the feces, varied greatly. It should also be emphasized here that in making determinations on rats less than about 60 days old special precautions are necessary to prevent coprophagy. But most albino rats have proven to be excellent subjects. Injections of test material have been given to some rats daily after the age of 15 days; to older rats, from 2 to 8 times daily for over a year (the oldest was 830 days); throughout pregnancies and even in the midst of deliveries, without mishap. Selected and properly handled rats do not defecate when material is injected and show that they are not otherwise disturbed by eating immediately afterward when hungry. Obviously, intractable rats were not kept for the more prolonged observations.

White mice. Only glass, silver, and gold balls were given to mice. Sometimes all test material was passed within 10 to 20 hours and no difference in the rates of passage of the various materials was then noted. Fast rates like these are probably normal for vigorous mice, but such mice were hard to manage and were soon injured. Tamer mice were apparently less vigorous. They passed all test material slower but glass was passed faster than the metals. One mouse repeatedly passed some metal first, then glass, and finally the remaining metal. After killing this animal, it was noted that all gold had left its funnel-like stomach before any glass. Part of the metal evidently passed through the entire digestive tract without being unduly delayed in the cecum but metal which became lodged in the cecum very likely left it only after all of the glass had passed. The difficulty of giving test material to mice without injuring them discouraged extensive work with this species. A periodic cecal stasis, but less than that seen in most rats, was suggested but not clearly demonstrated.

Example of tests. Mouse 5, female, given 11 blue glass balls and 11 gold balls. Passed within 9 hours, 8 glass, no gold; next 15 minutes, 1 glass, 2 gold; next 3 hours, 2 remaining glass, 3 gold; next 12 hours, 5 gold; next 12 hours, 1 remaining gold.

Birds. In the writer's earlier report, the opinion was expressed that gravel, on account of its specific gravity, evidently accumulated in the gizzard of birds because the gizzard is a dependent pouch, practically hanging from its orifices (the proventriculus and pylorus). In this study, the three pigeons that were observed were all males. One was normal while the other two had survived operations on the cerebrum for a considerable time but remained weak and ate little. The normal pigeon was observed for 12 days, the other two pigeons for 3 days each, and a laying hen for 15 days. All of the birds passed knots and rubber faster than glass or heavier material. In the normal pigeon, over 95 per cent of the knots were rubbed to fiber. A fair portion of fiber usually passed within 6 hours while a little passed only after 96 hours. The fiber retarded in passage is believed to have come mainly from knots that were retained longer in the crop. Rubber, even black rubber, was quickly reduced, apparently by both chemical and mechanical action. Nearly 50 per cent of the glass beads given to the normal pigeon were broken or pulverized in the gizzard. Some glass balls were also cracked. All metal was highly polished. Silver and gold were worn down. Steel was much corroded. The glass remaining in the gizzard presented a beautiful ground glass effect. Nothing like it was observed in any mammal. The passage of heavy material in general seemed to be determined more by its size than by its specific gravity, but occasionally some gravel, larger than any test material given, was passed. Only a little fiber from knots and a little broken glass were found in the intestines. The operated pigeons evidently had more sluggish motility of the gizzard but it was still powerful enough to rub up about 45 per cent of the knots and crush some beads within 3 days. One of the operated pigeons had only 3 pieces of gravel (spontaneously swallowed) and the other only 16 pieces in its gizzard. The normal pigeon had 40 pieces and two other normal pigeons, not otherwise observed, had 53 and 33 pieces of gravel in their gizzards. Apparently the operated pigeons failed to pick up much gravel as, with less motility, they should have had a greater retention than the normals.

In contrast to the normal pigeon, the hen passed from 90 to 95 per cent of the knots and pieces of rubber intact, but the others were clearly rubbed up mechanically. Some glass beads were also broken, but the proportion was small. The hen furnished better evidence than the pigeons that small heavy material passes out of the gizzard sooner than large material, even when it is much lighter. Thus small pieces of gold passed faster than large beads but knots and pieces of rubber nevertheless passed faster than small beads or small pieces of metal. The hen's gizzard contained 50 pieces of

gravel, including 3 pieces of glass swallowed spontaneously. An interesting finding was the presence of a considerable amount of gold in the duodenum and of silver in the jejunum. This seems to account for the fact that the hen ate little during the last 36 hours. Further observations on other fowls would be necessary to determine whether such intestinal stasis is typical, but a logical basis for it is seen in the fact that the duodenum of the hen, and also of the pigeons, consisted of a dependent loop folded upon itself. Passage of discrete particles, like the test materials, would also be restrained at the relatively sharp flexures. One of the weaker pigeons also had a few pieces of gold in its jejunum, which might indicate local stasis. No test material was found in the ceca of the hen although food residues were present.

Further work on man (part 2). Observations were made on the writer to determine whether the rate of passage of gold and silver varied independently of the rate of passage of light material, as in the monkey. For this and the study of related problems, differently marked pieces of gold and/or silver were taken daily for a period of 317 days; pieces marked the same way were not taken again until after all of that type were recovered. Five main periods in the daily intake of gold and silver may be distinguished: first, a preliminary period of 20 days in which the amount of metal taken daily was gradually increased; second, the period indicated in figure 1 in which 10 pieces of gold and silver, 10 glass beads, and 10 knots were taken; third, 116 days during which 25 pieces of gold and from 25 to 50 knots were taken; fourth, 132 days with 10 pieces of gold and 10 knots daily, and finally, 21 days with 10 pieces of silver and 10 knots daily. Altogether, 5589 pieces of gold, 530 of silver, 310 glass beads, and 6740 knots were taken during the 317 days.

All of the metal was recovered. Data obtained during the first 13 days did not indicate a striking variation in the rate of passage of the heavy metals, such as was seen in the monkey. Hence, the effect of changes in the diet was next observed. Here it will only be said that this reopened the previous question, namely, that of variations in the passage of gold and silver independent of changes in the diet. Consequently, more prolonged observations were made with the diet and the roughage intake kept more rigidly constant. As a result, some changes in the rate of passage or in the degree of stasis of gold were quite definitely indicated (fig. 4). These variations were not found to be accompanied by corresponding changes in any subjective symptoms. A complete explanation of the phenomenon did not suggest itself, but it seems evident that it is similar to the periodic stasis and the mass movements of metal seen in rats. Further studies on rats may clear up this problem.

One factor that tended to modify the passage of heavy metal in man, in a somewhat paradoxical way, was transient constipation. That is, constipa-

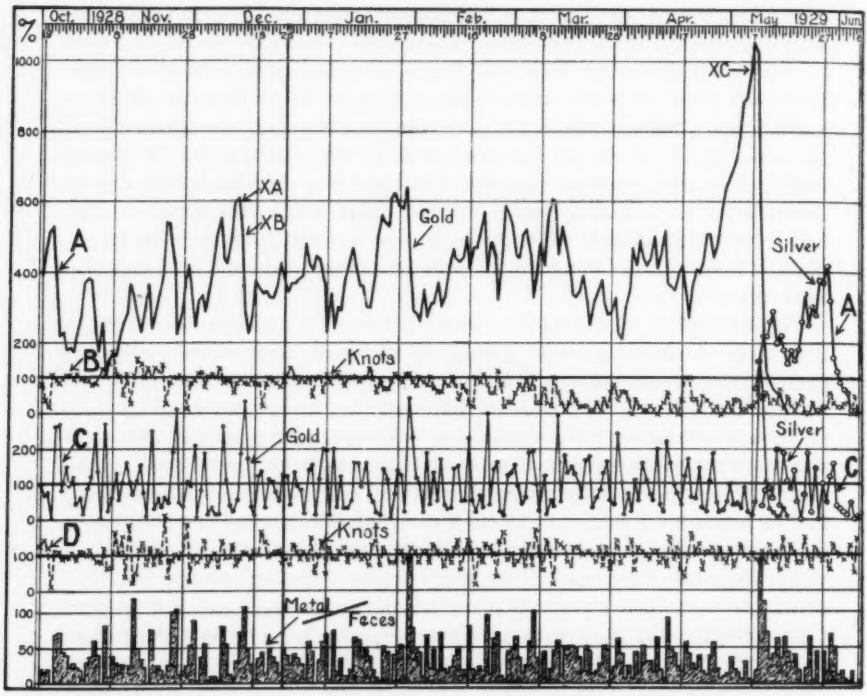
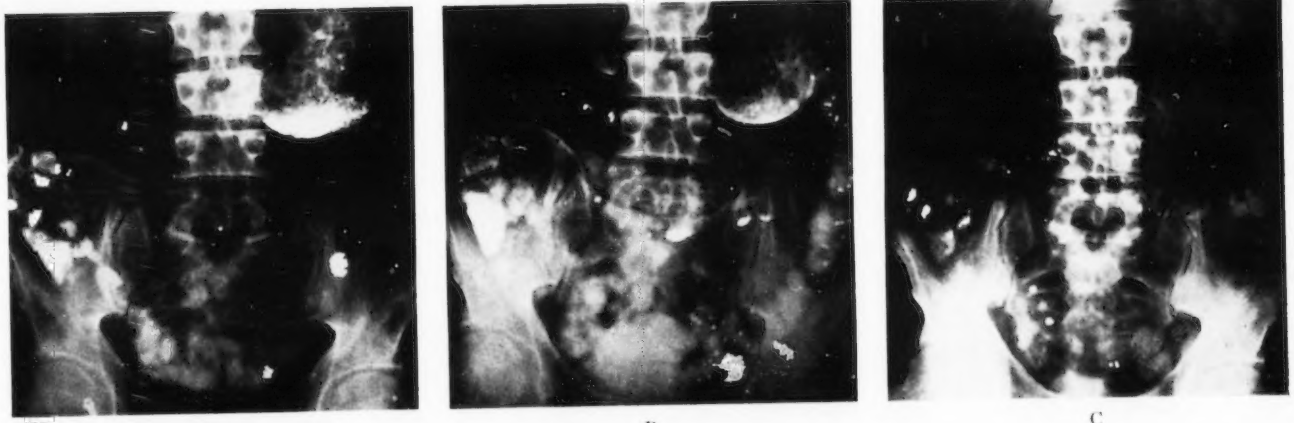


Fig. 4. Showing that the degree of stasis of gold and silver and its passage in man (F. H.) generally varied independently of the stasis and passage of knots, but some tendency toward an inverse relationship is also indicated. Curve *A*, *A*, shows the per cent (of the daily intake) of pieces of gold or silver that were retained in excess of the per cent (of the daily intake) of knots that were retained. *XA*, *XB*, and *XC* indicate when the roentgenograms shown in figure 5 were taken. Curve *B* represents the per cent (of the daily intake) of knots retained over 24 hours. Curve *C*, *C*, per cent of pieces of metal passed daily. Curve *D*, per cent of knots passed daily. The columns indicate the percentage of the daily intake of metal passed per 100 grams of feces. Twenty-five pieces of gold and 25 knots were taken daily until December 26th. Then 10 pieces of gold and 10 knots were taken daily until May 7th. Then 10 pieces of silver and 10 knots were taken daily until May 27th, inclusive. The diet and roughage intake were varied considerably before December 1st. Thereafter the amounts of food and roughage were kept fairly constant but occasional experimental changes were made in the order of intake of the major portions of protein, fat, carbohydrate, and roughage. The diet consisted of foods without fiber or freed from it. The roughage was a weighed amount of cellulose (modified Cellu Flour) mixed with petroleum jelly.



A B C

Fig. 5. Roentgenograms showing accumulations of gold test material chiefly in the cecum, colon, and terminal ileum of man (F. H.). A, taken December 13th, at *XA*, figure 4, shows 177 pieces of gold mainly in the cecum and colon. The speckled condition in the stomach is not due to the presence of any metal; it merely indicates unevenly distributed barium. The large mass of metal in the left pelvic region is evidently in the transverse colon and was probably in the cecum a day or two before when no x-ray observations were made. B, taken 24 hours after A, at *XB*, figure 4, shows that the cecum has emptied itself of most of the gold, which is moving across the transverse colon in masses. The pieces of gold distributed throughout the descending colon and rectum very likely came mainly from the large mass regarded as being in the transverse colon in roentgenogram A. Forty-six pieces of gold had passed and 25 were taken since roentgenogram A was made. C, taken May 6th, at *XC*, figure 4, shows 99 pieces of gold, chiefly in the cecum and terminal ileum and less in the colon. This evidently represents the usual sites of stasis better than A which was taken closer to the period of increased passage of metal per rectum. The small masses of gold indicated at the right of the lumbar region of the spine are in the small intestine (in the duodenum?).

tion, as indicated by a slowing down in the passage of knots, often led to a speeding up in the passage of gold and silver and *vice versa* (fig. 4). The chief sites of stasis of gold and silver were the cecum, colon, and terminal ileum (fig. 5). Constipation naturally led to a greater dehydration of the food residues in the colon and such residues would carry along the gold and silver better. It is also possible that transient constipation, by leading to a greater filling of the colon, eventually gave rise to more vigorous peristalsis which helped to clear out the heavy metal. The effect of a change in the consistency of the food residues on the rate of passage of heavier material was also demonstrated by adding India or karaya gum to the diet (fig. 1). Then, during two days when from 10 to 13 grams of gum were added to a diet containing about 1000 calories, glass beads passed as fast as knots, but on the third day when the diet was increased to over 5000 calories while the amount of gum added remained unchanged, the beads failed to pass as fast as the knots. This is also the reverse of what the change in food intake alone would do, as a diet of only 1000 calories, without gum added, nearly always led to an increased stasis of heavy material.

Among the 5589 pieces of gold taken during this investigation, the rate of passage of the individual pieces varied from 27 to 528 hours, or from 1 to 22 days. When 25 pieces of gold were taken daily, the average rate of passage of all gold taken on any day varied from 67.1 to 211.8 hours, and when 10 pieces were taken daily, it varied from 68.1 to 275.9 hours. With less gold taken, the rate of passage tended to be longer. Such a tendency was more clearly demonstrated on the rats. In making the transition from taking 25 to taking 10 pieces of gold daily, the smallest pieces of metal were selected (they varied from 1.5 to 2.0 mm. in the largest diameter). The 10 pieces of gold consequently represented a mass less than one-third of the mass when 25 pieces were taken daily. The question therefore arose whether a stasis of an equal mass of metal, that is, a percentage of the daily intake 3 times as great as when 25 pieces were taken, would occur. Figure 4 indicates that the percentage of gold retained was by no means increased to such an extent, but the unusual accumulation of gold toward the close of the experiment shows that increased retention was possible. The impression therefore is that a tendency on the part of the digestive tract of the subject to pass the metal at certain times was a greater factor in determining its passage than the amount of metal present.

Another question was whether heavy test material is deposited in the haustra or other dependent parts of the digestive tract in stratified layers, with the consequence that material taken later would tend to pass sooner. Alvarez and Freedlander suggested such an explanation of their observation that glass beads taken on the second and third days tend to catch up with those taken on the first. It was already stated by the writer (1924)

that, with regard to glass beads and knots, no such phenomenon was observed after the first 3 days, but it was observed then. It was never observed while gold and/or silver were taken. It could not be observed when mass movements of metal took place. When gradual clearing out occurred, curious variations in the sequence of passage of gold were often noted. But the results suggest that the sequence was largely determined by chance. If we assume that the accumulating material is shuffled more or less by local motor activity, before or during passage, this would account for the results.

If we next consider the amount of mixing of test material or of food residues that takes place from day to day, the data indicate that the heavier the material, the greater will be the extent of the mixing. For instance, during the period represented in figure 1, the average number of days that elapsed between the initial and final passage of the daily intake of knots was 0.84, in the case of beads it was 1.43 days, with silver 4.04 days, and with gold 6.54 days. The average rate of passage of these knots, beads, silver, and gold was 29.7 hours, 47.3 hours, 81.1 hours, and 123.5 hours, respectively. This shows that with the increasing specific gravity, the extent of the mixing of the test materials increased even more, proportionately, than the rate of passage. In the earlier report, it was suggested that such mixing of the food residues as appeared to take place, judging by data obtained with knots, was due to telescoping of the diurnal food residues upon one another in the colon. However, with over three full years of additional observation, a fairly clear separation or specific retardation of from 1 to 3 per cent of the knots has been seen frequently enough to convince the writer that some true mixing of food residues may occur, presumably in the cecum as suggested by Alvarez and Freedlander. Whether even this degree of mixing is normal, is still a question. Definite evidence of it could not be secured independent of some degree of constipation. Recently, for example, 100 knots were taken daily for 23 days to obtain more detailed data on mixing than one can secure when less test material is taken. Fifty-three and nine-tenths per cent of these knots passed during the first 24 hours, 43.8 per cent passed the second day, 2.2 per cent on the third day, and the remaining 0.1 per cent (2 knots) on the fourth day. One of the knots remaining over three days was evidently passed backward as a result of taking a barium enema for x-ray on one occasion during this period. The other instance of delay in passage beyond three days followed a bowel movement in which only one-half of the usual quantity of feces was passed. These data therefore indicates that very little mixing occurred under the conditions then maintaining, namely, one bowel movement daily and an intestinal rate averaging 27.8 hours. The bearing of the foregoing on the problem of intestinal toxemia seems obvious, and as Alvarez has apparently been guided since 1913 (Alvarez and Freedlander,

1924) by the erroneous data gained with glass beads (which show much more mixing than knots), this may help to account for his particular attitude concerning auto-intoxication.

Determinations of the intestinal rate with the simultaneous use of millet seed and knots were also made in order to evaluate Burnett's method (1923). This method consists of taking 50 cc. of French millet seed and then, by gross inspection of the stools, noting the initial and final rates of passage as indicated by the superficial presence of more than 5 seeds. In all of four such tests on the writer, the initial rates for the seeds and knots were alike but the final rate for the seeds was about 24 hours longer than the final rate for the knots. In sieving the feces, however, a few seeds were seen to be retarded as long as 72 hours after the last of 100 knots passed. In four other tests in which 100 French millet seeds and 100 knots were taken, the seeds passed slower than the knots. This confirmed data obtained earlier when common millet seed was used (table 1). In introducing the millet seed test, Burnett (1921) claimed that charcoal was retarded in passing through the digestive tract. But tests made with charcoal and knots by the writer showed neither initial nor gross final retardation of the charcoal. Moreover, when 50 cc. of French millet seed, 100 knots, and 1.5 grams of charcoal were taken together, the knots and charcoal passed in equal time while the final passage of the seeds was delayed as in the other tests. Burnett's method therefore is misleading rather than advantageous.

In this connection, it should also be noted that Burnett, with his method, finds a slow intestinal rate of 62 to 134 hours as normal. This, in spite of the fact that he adds the bulk involved in giving 50 cc. of seeds. In the writer's experience, millet seed was not only retarded in its final passage but 50 cc. usually slowed down the intestinal rate about 12 hours, very much as the 300 grams of glass beads, referred to in the opening paragraph, slowed down the intestinal rate. If Burnett's figures were corrected, the intestinal rate would be about 50 to 100 hours, which is very likely normal for the type of diet that he advises. Fifty cubic centimeters of seeds are as much bulk as, or more bulk than, 60 grams of barium sulphate. But such a quantity of barium, Alvarez and Freedlander regarded as a large amount, and they attributed the faster passage of beads taken with barium to its bulk. In this study, 25 grams or more of barium was observed to help sweep out heavy test material, although the passage of light material was slowed down. Obviously, this and the faster passage of glass beads noted by Alvarez and Freedlander was due to the fact that beads or metal would be carried along better by a heavy barium suspension or by barium meal residues than by the normal and lighter contents of the digestive tract. In some instances, a barium meal may also act as a specific laxative, just as the massing of heavy metal in the digestive tract of some animals

led to mass movements of the metal and diarrhea. Likewise, Tetelbaum (1926) noted that with a barium meal a patient's stomach appeared to be hypertonic while tests made with a balloon filled with a salt solution showed the stomach to be atonic. Tetelbaum believed that the difference in the consistency of the gastric contents caused the different motor responses. His further experiments did not support this explanation. Evidently the high specific gravity of the barium meal and not its consistency led to an increased gastric tonus in the type of subject used. It also seems to the present writer that a considerably increased tonus or motor response accounts for the not infrequent perforation of ulcers after taking heavy barium meals.

Some intestinal rate tests were also made with psyllium seed because it is finding increasing use as a laxative and for comparison with millet seed. Psyllium seed was somewhat faster in passing than millet seed, perhaps because the swelling of the outer layer makes it relatively lighter, but it passed slower than knots (cellulose). Carmine was not tried further after finding that it caused nausea when taken during a fast. Alvarez and Freedlander also pointed out that it gave rise to diarrhea in pups.

At the close of the foregoing experimentation, a complete gastro-intestinal x-ray examination of the writer was made in the division of roentgenology of the department of medicine. According to the report of Dr. P. C. Hodges, no abnormality was found. During the experimentation, 9 stereoroentgenograms were made to determine the location of the metal test material. In connection with all but 2 of these 9 occasions, barium was used in varying amounts (12 to 35 gm. daily). As the metal was usually massed in the cecal region or in the colon, the possibility that some stasis of metal may have occurred in the duodenum was not given sufficient attention. The pictures in figure 5, as well as others which were taken about 15 hours after last swallowing metal, suggest some duodenal stasis. This, however, may be normal for the duodenum. Further observations, under circumstances that would better favor outlining of the duodenum, would be necessary to clear up this point.

General observations and comments. As the question of intestinal toxemia is naturally related to the intestinal rate problem, it was hoped to obtain data simultaneously on both subjects. The Bergeim test for putrefaction was tried but this proved to be of no value in its trial on rabbits, as reported elsewhere (Hoelzel, 1929). Some evidence of the degree of putrefaction was nevertheless apparently indicated by the degree of blackening that silver underwent in passing through the digestive tract. Usually, the degree of blackening was proportional to the length of stay in the digestive tract and was also related to the character of the diet. Instances of greatly increased blackening were noted in rabbits that became obstructed. Then silver blackened quickly and deeply as high as in the

duodenum, but not in the stomach. Otherwise little blackening of silver occurred even in the colon of rabbits. Sometimes only a little blackening of silver took place in the cecum and appendix of rabbits and in the cecum of rats in spite of prolonged stay and even at times when silver blackened quickly in the small intestine and colon. This may mean that conditions in the cecal region of these animals are normally such as to prevent much putrefaction. However, as silver can blacken by contact with some foods independent of putrefaction, this test, like the Bergeim test, is not reliable under all circumstances.

Gold test material which was unduly delayed in passing was often covered with a red, brown, or greenish film or encrustation. This was apparently due to a bloody or fibrinous effusion into the digestive tract. The encrustation was heaviest and dark brown in obstructed rabbits that also had tarry feces. It developed only in the duodenum in the rabbits. Not all gold remaining for the same length of time showed a similar degree of film formation or encrustation. Gold remaining in man more than 8 days often had a red film. The film was usually heavier and darker on some gold passing later and a greenish film was present on nearly all gold remaining over 15 days. Such observations suggested the use of heavy metal for experimentation with prolonged mechanical irritation of the digestive tract. However, even after giving rough pieces of gold and silver daily to rabbits for 4 months and to rats for 8 months, no serious lesions have been produced when obstruction was avoided. The ability of healthy and well-nourished animals to withstand such apparently drastic mechanical irritation of the digestive tract is astonishing. But tests are being continued on rats with larger amounts of metal in the belief that some lesions will eventually develop. The lesion (ulcer) that developed in the colon of rat 13 is regarded as a "spontaneous" manifestation—a consequence of the condition of the animal rather than the result of mechanical irritation.

The observations in this study did not indicate that the motor mechanism of the mucous membrane of the digestive tract is responsible for the specific retardation in the passage of the heavier materials. Forssell (1928) expressed the view that the mucous membrane, by virtue of its own motility, tends to elevate itself around solid particles and thus holds them and retards their passage until they are more or less digested away. Apparently, some of the best evidence of such action (the imbedding of fragments of food, bone, and hair) was noted by Forssell in dogs, where the mucous folds are prominent. But it is precisely in dogs that heavy metal is passed relatively fast. The stasis of heavy material in the various animals occurred mainly in dependent parts of the digestive tract or in parts with feeble motility. The formation of mucous folds, however, may play a minor rôle in determining variations in the passage of solid particles. But

it would seem that such folds might be quite as effective in retarding materials like knots and glass beads as in retarding the heavier pieces of metal.

Among the general impressions, a relation between the intestinal rate and the probable metabolic rate of the animals was seen. Young rats, for instance, had less intestinal stasis than older rats. Males had less stasis than females. Small animals also had a faster intestinal rate than larger animals—mice being faster than rats, monkey faster than man, and the normal pigeon faster than the hen. But this question would be better settled if similar differences were noted between dogs of small and large breeds. A relation between the general vigor of the individual animals and their intestinal rate seemed quite obvious, although exceptions were noted. An inverse relation between the general activity and the intestinal activity was also often seen. In such cases, however, the less active animals were perhaps less active because they were less nervous.

At any rate it would seem that the simple methods here employed deserve a wider application in studying the mechanics of the digestive tract of intact animals and also the relation of intestinal activity to other factors. There is a need for a more suitable material than knots or rubber for determining the normal intestinal rate in small animals but test materials of glass and metal also yield valuable data. The amount of test material, especially of heavy material, to be given to different animals, for obtaining data that can be directly compared, still remains an open question. Improvement in the technique of giving material so as to avoid undue disturbance of, or injury to, guinea pigs and mice is also highly desirable.

The possible clinical value of tests of the motor efficiency of the digestive tract by the use of heavy material still remains to be determined. The results in this study showed that heavy test materials tend to reveal changes in motility much easier than light test materials. Silver would probably be heavy enough to reveal any serious alterations in man. But perhaps the first objection to the use of heavy metal would be that it might become lodged in the appendix and give rise to symptoms. Scott's report of six cases (1928), with from 1 to 8 lead shot in the appendix and with symptoms regarded as being due to the presence of the shot, came to the writer's attention just before he began taking metal daily. Consequently, only small amounts were taken at first and increases were made only after close attention failed to reveal unusual symptoms. The amount of metal taken was later decreased because the smaller amount seemed to be sufficient for the purpose of the experiment. The appendix of the writer was never visualized and it is not known whether any metal ever entered it. But the view that something like lead shot in the appendix could be the direct cause of symptoms is considerably weakened by Sonnenfeld's accidental observation of an instance (1926) in which a young woman had 95 lead shot (some with sharp edges—total 24 grams) in her appendix, which

was large but otherwise normal in all respects. As this woman was the daughter of a hunter she very likely harbored lead shot in her appendix for years without symptoms. Examination because of uterine trouble led to the discovery of the mass of shot.

However, tests with lighter material could hardly be objected to in the same way. The clinical use of a uniform light test material would seem to be indicated in cases like one reported by Oehnell (1927). In that case an extreme irregularity in the order of passage of different materials was the chief evidence of a pathologic condition. But the extent of the irregularity was undoubtedly not as great as Oehnell apparently supposed, for his evidence was based upon observations of the passage of materials such as barium, seeds, and different food fragments which would normally pass at different rates. Moreover, the giving of an enema at one time may have upset the order of passage and radical changes in diet evidently further complicated the results. However, a considerable irregularity in the order of passage of a uniform light test material, with conditions kept fairly constant, would more definitely indicate a disturbance in the gastro-intestinal motor mechanism. A relatively light test material that would be opaque to the x-rays would also be available if one used small balls of rubber impregnated with opaque material or small hollow balls or capsules of silver, either sealed or filled with cork or paraffin. Such test material might be useful in trying to locate sites in the digestive tract with abnormal stasis or segments with partial obstruction where a powder like barium would pass too easily or might be washed through. But here again tests on normal subjects would be necessary first to determine the normal range of variability in the passage of such material.

SUMMARY

The rate of passage of various inert materials through the digestive tract of 16 rabbits, 7 guinea pigs, 4 dogs, 2 cats, 50 albino rats, 8 white mice, 1 monkey, 3 pigeons, 1 hen, and 1 man was observed. The test materials included rubber, cotton thread (knots), seeds, glass beads, aluminum, steel, silver, and gold. Rates of passage more or less proportional to the specific gravity of the test materials were found, the heavier materials passing slower than light material. The rates of passage also varied considerably in the different species and individuals.

The main sites of stasis of the heavier materials were determined in some species. In rabbits, the stasis occurred most regularly in the duodenum and to a lesser extent in the colon, appendix, cecum, and stomach. In guinea pigs, most heavy material was retained in the stomach and less in the cecum. In the rats, the cecum was the main site of stasis and much less occurred in the stomach. The pigeons and hen retained most of the heavy material in the gizzard and the hen also showed some stasis in the

duodenum and jejunum. In man, heavy materials tended to accumulate mainly in the cecum, colon, and terminal ileum.

The stasis of gold was so great that intestinal obstruction could be produced with it in rabbits, but not in guinea pigs, rats, or mice.

Variations in the rate of passage of heavy test materials largely independent of changes in the passage of light material were noted and are partly explained by the observation that heavy materials tend to pass in masses when they pass at all.

A relation between the gastro-intestinal motor activity of female rats and their fertility, the viability of their young, and the sex ratio in their litters was seen. Other observations, tests, and relationships are also mentioned and discussed.

A large part of the foregoing investigation would have been impossible without the convenience of living in the laboratory. For this, particular thanks are due to Doctor Carlson.

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THE RESPONSE OF NERVE TO OXYGEN LACK[1]

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In an earlier paper (Gerard, 1927a) on the effect of asphyxia on the heat production of nerve during activity, some preliminary observations of action potentials were included; and it was proposed to analyze the mechanism of asphyxiation by means of further action potential studies. Other observations I made at that time indicated that the presence or absence of oxygen had a marked influence on injury potentials and on the shape and even the sign (positive or negative after-effects) of the action potentials in frog nerve. In the present experiments the study of these phenomena has been carried further, and in the course of the work a number of unanticipated effects have come to light. In the last two years, Furusama (1929) and Amberson and Downing (1929), in Hill's laboratory, where the above studies were carried out, have elaborated certain phases of these problems, and Gerard and Forbes (1928) have further studied positive and negative after-effects in the isolated nerve. A recent paper by Heinbecker (1929) has also dealt with asphyxia of nerve.

The thermal and electric response of a nerve kept in an oxygen-free atmosphere gradually falls to zero. Although it was calculated (Gerard, 1927a; Hill, 1928) that oxygen dissolved in the nerve should diffuse out or be used up within a few minutes, the fall of activity persists for hours. Since it was certain that the nerve required oxygen for the completion of chemical changes involved in activity (Gerard, 1927b) the existence of an "oxidizing reserve," sufficient to supply its needs during this time, was hypothecated (Gerard, 1927a, b). The chemical behavior of nerve during asphyxiation (Gerard and Meyerhof, 1927) has lent support to this view and it has been possible to construct a series of equations fairly accounting for the known changes of activity, equilibration, and asphyxiation of nerve (Gerard, 1927c).

Of considerable interest from this viewpoint is the question of just how the gradual decrease in activity during asphyxia is brought about. Since the total response to a tetanus is recorded, there appear three ways, not mutually exclusive, in which this response might be gradually lessened.

[1] Reported at the XIII International Physiological Congress, Boston, 1929.

1. Individual fibres might become blocked at different times; 2, the refractory period might progressively lengthen so that an increasing number of the stimuli fail to evoke responses, and 3, the single responses of individual fibres might be gradually depressed—as in the early stages of narcotic action. It was the prime purpose of this research to assign a quantitative value to each of these factors, and the results obtained demonstrate that all play a rôle. The appearance of other effects, however, has rendered an exact numerical statement rather dubious.

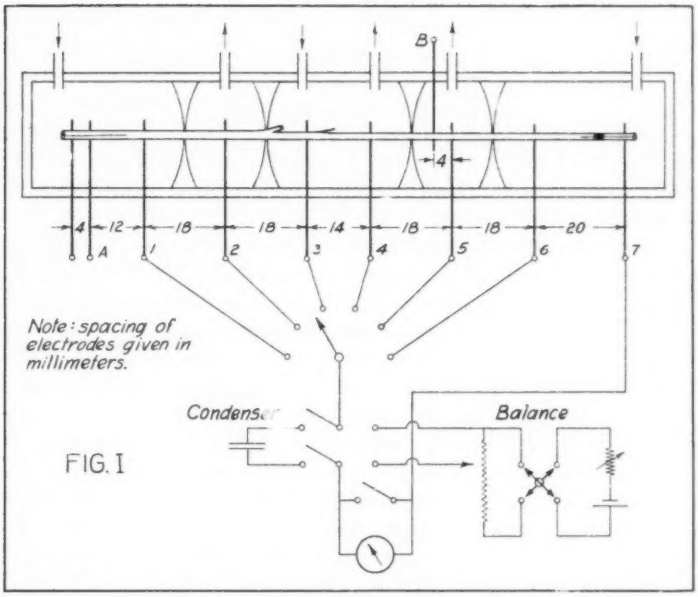

Fig. I

METHODS. *a. Nerves.* Sciatic nerves of the grass frog and bull frog taken at all seasons, and the unbranched peroneal nerve of the dog have been used. The nerves were rapidly dissected, with the usual precautions against stretch or other mechanical injury, tissue tags or blood clots, and with branches cut far from the main trunk. The isolated nerve was at once laid on electrodes in a moist chamber, and although only moistened with Ringer's solution (unbuffered) sufficiently to insure good contact, it remained moist and usually in excellent condition for 24 hours or longer. Experiments were made at room temperature, which varied at different times between 19° and 28°C.

b. Chamber. For the bull frog and dog nerves a large vulcanite chamber

with five compartments (fig. I) was used; for grass frog nerves a smaller one with three. Ringer soaked filter paper covered the floor of each compartment and gas inlet and outlet tubes were supplied to each. The dividing partitions were only ½ mm. thick at the center and were here each cut by a vertical slit half way down, to accommodate the nerve. When the nerve was in place, the slits adjoining the middle compartment were sealed with vaseline or a mixture of vaseline and kaolin and the whole covered by a glass plate on a vaseline seal. A minimum of material was used about the nerve in closing the partitions since a mass of vaseline covering several millimeters length of nerve would cause parial asphyxia of the center of such a stretch; and it was not entirely simple to make the seal airtight. Careful blotting of the nerve before applying the vaseline was necessary, and manipulation was continued in all cases until the compartment would maintain a pressure of 50 mm. or more measured on a water manometer. Compartments *1* and *2* and *4* and *5* were usually left in communication. The whole chamber was thinly coated with paraffin to insure against electrical leaks.

In compartment *1* were two stimulating electrodes *A* and lead 1, in the second compartment lead 2, in the third leads 3 and 4, in the fourth lead 5 and an adjacent electrode which, with 5, could be used as stimulating electrodes *B*, and in the fifth compartment lead 6 and lead 7 from the injured end. Action currents were led from 1–6 by a dial switch and directly from 7. The nerves lay ordinarily with their central end on stimulating electrodes *A* and were crushed 2 to 3 mm. above lead 7. All electrodes were of thick silver wire and, whether used with a condenser in circuit or not, were freshly coated with silver chloride for each experiment. A wire with small silver plates soldered at appropriate intervals to fit down between electrodes was very convenient for this coating. No electrode was placed within 15 mm. of another nor within 9 mm. of a partition. The total length of nerve required was over 12 cm.

For the grass frog nerves, the small chamber with three compartments was used. The electrode arrangement was similar but only four leads were taken from the side of the nerve, one in each of the end compartments and two in the center one.

c. Gases. Moist oxygen was kept bubbling slowly through all compartments except when replaced in the center one by nitrogen. It was led in through the outside tube of the end compartments and out through the second and fourth, thus insuring a steady stream of oxygen towards but not into the central compartment. A separate tube led oxygen into the center compartment and a simple adjustment allowed the substitution of nitrogen for oxygen, or the reverse. The outlet dipped into several millimeters of water to prevent back diffusion.

Nitrogen obtained from the Air Reduction Co. containing not more

than 0.3 per cent of oxygen was used. This was regularly further purified by slow passage over heated copper gauze, though experiments without this extra precaution showed no different results. The gas was led through glass tubing with a minimal amount of rubber pressure tubing at the joints, and through several wash bottles to moisten and cool it. The rate of bubbling was kept approximately constant (one bubble every few seconds), as gross changes in gas flow caused variations in action potentials.

d. Stimulation. Tetanizing stimuli delivered by a Harvard coil, even when the buzzer contacts were of platinum, were not sufficiently regular to give uniform responses. Entirely satisfactory results were obtained with steel hack-saw blades vibrating a point into a mercury pool, and under the control of an electro-magnet in the interrupted circuit (Bernstein interrupter). Over a wide range of adjustment of the contact (such as raising or lowering the point) between point and mercury, constant responses were obtained. The primary of the induction coil was in this circuit, with a

TABLE 1

CAPACITY	ACTION POTENTIAL
mF.	*mm.*
11	8
6	40
4	85
2	117
1	126
0.5	135
0.2	137
0.1	95
0.05	76

two volt storage battery, and the vibrator was kept going through the whole experiment. The nerve was stimulated by closing a two pole key on the leads from the secondary coil. The secondary at 12 to 13 cm. and horizontal usually supplied slightly supramaximal stimuli.

It is necessary to connect a condenser across the point and the mercury into which it vibrates, to prevent sparking and consequent irregularities and oxidation of the mercury. In the past a 10 M.F. condenser has been used, but it was found that the stimulating effect of the secondary shocks is profoundly influenced by the size of the condenser across the primary interrupter. Table 1 shows this effect on one preparation, everything being maintained constant except the condenser across the spark gap. Maximum action potentials were obtained with a 0.2 to 0.5 M.F. condenser, but some sparking appeared when the capacity was reduced below 3 M.F. and became quite marked with only 0.5 M.F. As a final compromise, a 2 M.F. condenser was used, which gave a very faint spark and required only a slight increase in shock strength to give maximal responses.

Two such systems were used, one giving 304 (make and break) shocks per second, the other 88. The nerve was tetanized for a second every 30 seconds, this regular repetition being usually rigidly adhered to so as to avoid equilibration effects (Gerard, 1927a, b). Starting with fast tetanization, action potentials were led successively from electrodes 1 to 6, and then again with slow tetanization, so that an observation at one frequency from one lead was repeated every six minutes. The regularity of response when this schedule was adhered to and their scatter when it was violated attest its importance.

e. Recording system. For studying the integrated response of a nerve to a rapid succession of stimuli, a slow recording instrument has many advantages; and in these experiments a sensitive moving coil galvanometer (1 mm. = 10^{-10} amp. at 5 meters, period 3 seconds) was used throughout, its deflections being read directly from a scale. In some experiments the injury potentials from the nerve were balanced in the usual fashion, but this involved a readjustment of the balancing potential at each electrode, and temporary polarizations, and in most experiments a 10 M.F. condenser was inserted in the circuit (fig. I). The galvanometer was shorted when the switch was turned from one electrode to another, while the condenser charged to the new injury potential, (if not shorted the deflection gives a measure of the resting potential between the two electrodes) and the short was then opened, leaving the galvanometer on "open circuit" through the nerve and the condenser of "infinite" resistance. Any change of potential in the system, as when one lead becomes less positive due to activity of the nerve, causes the condenser to charge or discharge and send a momentary current through the galvanometer, which is read ballistically. Since a 10 M.F. capacity is slow in changing its charge, especially through a high nerve resistance, and the galvanometer slow in responding, rapid oscillations of potential will be smoothed out and a deflection result which directly measures the average potential reached.[2] This insures an integration of

[2] The effectiveness of any quantity of current in producing a deflection is greater the earlier in the deflection period it passes through the galvanometer. Here the greatest current flows early in the deflection period (condenser changes logarithmically) and it is most effective at this time. Through 50,000ω, a 10 m.F. condenser requires 1.5 seconds to reach 95 per cent of its full charge, through 10,000ω only 0.3 second. This means that for a longitudinal resistance of the nerve of 10,000ω, 95 per cent of the total current will have affected the galvanometer, for a 50,000ω resistance only about 60 per cent.

In a series of experiments to directly test the system, a constant quantity of electricity was discharged through the galvanometer from a variable capacity through a variable resistance. With 0.1 or 1.0 m.F. the deflection was independent of the series resistance between 10,000 and 100,000ω. With a 10 m.F. capacity the deflection became less as the resistance rose above 10,000ω, but even with 50,000ω deflections were nearly 80 per cent of the maximum, instead of 60 per cent. When

all potential changes during the ballistic "utilization period" of the galvanometer. Thus ten nerve impulses early in this period would give ten times the deflection of one, a prolonged action potential more than a short one, etc. The use of the condenser makes balancing the injury potential unnecessary, minimizes polarization, and practically eliminates changes in the longitudinal resistance of the nerve as a factor determining the magnitude of the recorded responses. Numerous controls of the reliability of such a system have been made, and it may be pointed out that the results obtained are alike whether the balanced circuit or the condenser is used.

f. Method of analysis. As long as the response at lead 5 or 6 to stimulation at A remains constant, the total number of active fibers and the impulses they carry are not changing; as it falls the ratio of any particular response to the initial ones represents the percent of fibre-impulses still arriving. The comparison can be made more accurate and any gradual failure of the nerve allowed for if the response at lead 6 to *A* stimulation (above the asphyxiated stretch) is compared to the same for *B* stimulation (below the asphyxiated stretch). A fall of fibre-impulse value means either blocked fibres or a prolonged refractory phase in the nitrogen stretch with fewer impulses per fibre passing through. This second factor is studied with the aid of fast and slow tetanization. A lengthening refractory period must cause a decreased response to stimuli at 3.3 σ intervals earlier than to those at 11.4 σ intervals.

Changes in the average size and form of the action potential for a single impulse in a single fibre can be estimated by comparing the responses at leads 3 and 4, in nitrogen, with those at 5 and 6 below the asphyxiated stretch. A decreased response in the exposed region with no change or a smaller decrease in the control region below must signify depression of conduction in the exposed region. Such a decreased response means a decrease in the area of the time-potential curve of activity, since it is the integrated potential change that is measured, and the decrease may be in magnitude or duration of the action potentials. An increased response would similarly represent a higher potential or a greater duration of an unaltered potential. Again, a comparison of the responses to rapid and

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a nerve was used, instead of a resistance box, this test showed that the discharge from the 10 m.F. condenser was a bit over 80 per cent as effective as the same quantity of electricity discharged from a 1 or 0.1 m.F. one.

A considerable change of nerve resistance, however, causes little change in deflection for a given voltage. For example, with a nerve crushed below lead 3, responses to stimulation at *A* from leads 1 and 4 were 134 mm., from leads 1 and 7, 132 mm. The potential in both cases was the same, the nerve resistance between 1 and 7 over twice that between 1 and 4. Although, therefore, only a fraction of the total current acts upon the galvanometer this fraction is approximately constant over a considerable latitude of resistance change in the nerve.

slow tetanization gives a clue as to which effect is present. An increased potential with no change in time should increase the fast and slow responses proportionately. An increased duration should increase the slow responses relatively more than the fast, unless the "tails" of the action potentials are completely additive (Amberson and Downing, 1929, find only partial addition), since a longer portion of each persistent potential will come to expression before smothered in the next action wave. The decrease of a positive after-effect would, of course, act similarly to an increased duration of negative potential. Reference to figure III may make the matter clearer. The methods and interpretations applied in studying the effect on nerve heat production of changing frequency of excitation (Gerard, Hill and Zotterman, 1927) are also of interest in this connection. From those results it is clear that the intensity of the nerve's response to a stimulus is highly dependent on the time elapsed since its previous response. A prolongation of refractory period and a decreased impulse response in nitrogen might be, from this viewpoint, hardly more than two expressions of the same change

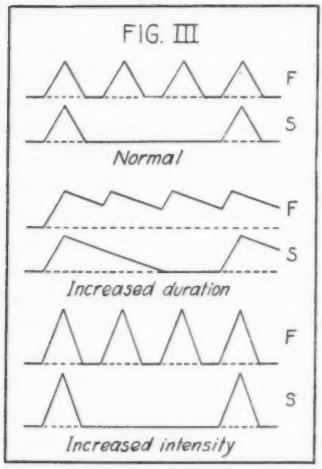

Fig. III

RESULTS. 1. *Initial values.* It is of some interest to compare the responses at different electrodes to different stimuli at varying times and for several types of nerve.

In general, the green frog nerves give deflections over twice as great as those of bull frogs or dogs, table 2. It seems probable that the higher values with the small frogs result from a thinner and less resistant perineurium, though different sized fibres may be a factor. A similar but more marked difference in the two kinds of nerves is shown in the response to oxygen after asphyxia, when the rise in action potential of green frog nerve may be several times that of the bull frog nerve.

The lead near the crushed end (15 mm. away) is regularly depressed to $\frac{2}{3}$ or $\frac{1}{2}$ the values of the others (see Davis and Brunswick, 1927) which are all of the same general magnitude. Since the nerve resistances between leads 1 and 7, and leads 5 and 7 are widely different but the galvanometer response to activity is approximately the same for each pair, this affords further evidence that, with the condenser circuit, the deflection directly follows the potential change and is largely independent of nerve resistance.

Although the responses at leads away from a crushed end are roughly

alike, in any one experiment the responses at individual leads often show wide variation. To a certain extent this was found to depend on the special relation of the various branches to the leads. For example, the main thigh branches leave the bull frog nerve between electrodes 2 and 3, and electrode 2 usually gives lower values. Still, variation of responses is found along the dog's peroneal in the absence of branches, and local condi-

TABLE 2

DATE	NERVE	TEMPERATURE	TIME SINCE DISSECTION	RESPONSE IN ARBITRARY UNITS											
				Fast tetanization Lead						Slow tetanization Lead					
				1	2	3	4	5	6	1	2	3	4	5	6
1928–29		°C.	hrs.												
August 9	Green frog	26	½	(a) *130	105	105	75			50	40	45	40		
				(b) *105	90	90	58			45	45	45	35		
			1	90	80	80	55			45	40	45	35		
December 6	Bull-frog	22	1	50	50	50	45	55	31						
December 28	Green frog	24	1	240	220	270	110			50	50	60	21		
March 25	Bull-frog	23	2	27	33	35	48	54	59	8	11	11	22	22	23
			28	38	24	23	31	32	16						
April 20	Bull-frog	22	2	35	30	26	28	35	24						
			4	34	25	24	24	37	23						
			24	47	11	34	29	32	15						
April 26	Bull-frog	26	½	72	39	65	70	71	10						
			2½	64	42	65	72	65	9						
May 18	Dog	25	2	42	41	34	37	28	26						
June 21	Dog	27	4	24	23	24	23	27	22	12	10	10	9	11	7

* (a) Stimulation repeated every 20 seconds; (b) stimulation every 30 seconds. Note equilibration effect of frequent repetition is more marked for rapid than for slow tetanization. Other values in table are for repetition at 30 second intervals.

tions under the electrode, amount of sheath, tissue tags, droplets of solution, etc., must be of importance (see also Davis and Brunswick, 1927).

These irregularities make the quantitative evaluation of changes somewhat arbitrary. If electrode 2 gives an initial response of 55 mm. and 3 of 40 mm., and under new conditions they give respectively 110 and 90, which has shown the greater change? The absolute increase of 2 is more than of

3, the percentage increase of 3 is greater than 2. In general the latter is the more satisfactory measure and is the one used here; but if, in the above example, lead 3 is 15 mm. less than 2 by virtue of some local accident and the axone activity is alike under each, the percentage change may give an unfair comparison.

A further interesting variation appears when the responses at one electrode to fast and to slow tetanization are compared. The ratio $\frac{\text{F (fast response)}}{\text{S (slow response)}}$ is roughly constant through the series at 2.5–3.0, but may differ irregularly from 2 to 3.5 at different leads from the same nerve at about the same time. It is worth noting that though the ratio of the frequencies used is 3.5, the ratio of responses is definitely less, sometimes only half so great. This figure, like that of Gerard, Hill and Zotterman (1927) $\left(\frac{F}{S} = 1.6 \text{ for } \frac{300}{90}\right)$ shows that at the higher frequency the nerve has not been able to recover so fully between responses as at the lower one or that considerable potential normally persists for more than 3σ after an action wave. Probably both effects occur. The higher temperature at which the present series was carried out accounts for the higher F/S ratio.

The responses for any electrode and stimulus change in the course of time (table 2). Usually all show a regular decrease, rapid at first and then very slow, but not infrequently the values at some electrodes fall and at others rise during the same time. The F/S ratio also tends to fall slowly with advancing age of the preparation.

The rapid early fall of responses is not due to dying of the nerve but rather the reverse, since it depends on the "crush sealing over" and allowing action potentials to reach lead 7 and so partly cancel responses at the upper electrode. A fresh crush or scald proximal but not distal to the original one restores the initial values, and when these again fall a third injury again does so. This point is of some importance in connection with the changes produced during asphyxia. The slow fall, between 1 or 2 hours and 24 or more, seems to be a true dying of the severed nerve cells. This fall is seldom beyond half of the early values and many experiments have shown no fall over night, or even an occasional rise. Even with a fall in action potentials of nearly half, the threshold of excitation has often shown no increase.

The responses at lead 6 for stimulation at *A* and at *B* are alike, or are a little less for *A* stimulation than for *B*, indicating some blocked fibres between these two points. The usual agreement between these values, even with supramaximal stimulation, indicates also that shock escape is not affecting the galvanometer. This is further certified by two regular observations. 1. As the shock strength is increased steadily from minimal

to strongly supramaximal, the observed deflections at each lead rise rapidly to a maximum, which is reached at all at the same stimulus strength, and are increased not at all or slightly (probably due to sympathetic fibres) with further increase in strength of shock. 2. When a stretch of nerve is blocked by asphyxia (which does not destroy its structure as does a crush) no deflections are obtained from leads below it.

For the other leads, comparison of the responses to tetanization at *A* and at *B* shows some interesting variations. When a stimulus is applied to a nerve between the intact and crushed end leads, as for stimulation at *B* and leads from 1 to 4 and 7, the situation becomes complicated by shock escape. A slow galvanometer leading from two uninjured points of an unbranched nerve should give no response when a region between the leads is rapidly tetanized with make and break shocks, since action currents would be diphasic and the induced currents are equal and opposite. Actually, however, a unidirectional current does flow and the galvanometer deflects, the direction reversing when the stimulating electrodes are reversed. This is the Fleischl effect, observed over half a century ago (1878) and rarely considered since. (See, however, Ebbecke 1922.) It indicates rectification due to unequal polarization in the two directions of flow, probably related to the different forms of make and break currents; and recalls the rectification observed with unequal or dirty electrodes (Hill, 1913).

When the action currents are not fully diphasic, due to injury at one end of the nerve, they produce a given deflection to which the effect of the stimulating current adds or subtracts. Both values are simply determined by taking deflections with the secondary leads first in one direction and then reversed. Halving the difference of the two deflections gives the stimulating current, and the true action current is given by their average. The same discussion applies to potentials when recorded with a condenser in circuit. Ordinarily the Fleischl effect is not over one-fourth of the action potential. Since it is dependent on polarization it affords some measure of the state of the cell membrane, and, as one might anticipate, decreases as the nerve dies. It is also decreased, reversibly, during asphyxia. Ebbecke (1922) reports a decrease of the Fleischl effect in nerve as a result of stimulation with strong shocks and, especially, on tetanization, which he interprets as indicating lowered membrane resistance and polarizability. This sensitiveness to the state of the nerve, incidentally, affords evidence that the effect is a biological one and not an electrode artifact. The nerve sheath may, of course, contribute to the polarization.

Another factor modifying responses to stimulation at B is the branching of the nerves. At *A* all fibres are stimulated and, although groups successively branch off from the main trunk, all fibres in contact with any electrode become active. At *B* only the longer fibres are excited, those

having branched off above this level remaining inactive and serving as shunts across the active ones (see Wade, 1924; Osterhout and Harris, 1929). The response at lead 4 is consequently slightly more than 3, with very few fibres leaving between them, and responses are similar for leads 2 and 1. Between 2 and 3, however, the large branches emerge and the anticipated sharp drop in galvanometer response is observed on passing from 3 to 2.

For stimulation at either *A* or *B* action potentials can be obtained from two leads on normal nerve with no crush between them. The magnitude is greatest when large branches lie between the two leads and the effect probably depends on this branching. Table 3 contains the results of one such experiment and its analysis is given to illustrate several of the points so far discussed.

TABLE 3

LEADS (LOWER ONE CONSIDERED CONSTANT)	RESPONSE TO STIMULATION AT	
	A	B
1–2	−25	+2
2–3	+8	+17
3–4	−4	−6
4–5	−7	−20
5–6	−11	
6–7	−11	−12
	sum (1–7) −60	−19
1–7	−47	−19

See text for explanation.

It will be noted, table 3, that the action potentials between any two electrodes are about the same for stimulation at *A* or at *B*, except when electrode 2 is one of the leads. It is just below 2 that the large thigh branch leaves the main trunk. Aside from the small action potentials, due possibly to differences in the nerve sheath at various points and independent of the point of stimulation, the following factors account for the findings.

With stimulation at *A* all fibres are excited. At lead 1 full sized potentials develop in each; at 2, because of proximity to an injury (the cut branch), potentials are much depressed in many fibres and the average change is less than for 1. Lead 1 therefore becomes negative to 2. At lead 3 those fibres which branched at 2 are out of the picture, leaving mainly fibres carrying full sized potentials—so this also shows a greater negativity than 2. Potentials at 5 are greater than at 6, near the crush, and at 6 greater than at 7, beyond the crush.

At *B* only the long trunk fibres are stimulated. Since there are no sig-

nificant branches below lead 3, potentials between leads 3, 4, (5), 6 and 7 are the same as for stimulation at *A*. At 2 and 1 there are present a large number of inactive fibres that leave in the branch below 2, and which act as shunts across the active fibres. The change at 3 is therefore greater than at 2. Since the same number of uninjured fibres are active at 2 and 1 and are shunted by the same number of inactive ones, there appears no significant potential difference between them.

It may be well to point out here that, with the usual connections of the galvanometer to a distal lead from the injured end and a proximal one from an uninjured portion, the deflection obtained on tetanization indicates a negative change of the proximal lead relative to the distal one. This is called throughout a negative deflection. A positive deflection indicates the reverse. No deflection, of course, means either that no potential change occurs under either lead or the same one under both. Since ordinarily complete absence of response at a crushed end is not attained, a zero deflection via leads from an injured end and intact side is not necessarily a critical value. This is amply illustrated by the records during asphyxiation of nerve.

With the condenser circuit described, a continuous regular tetanization leads to a new average potential on the condenser. The galvanometer gives a ballistic negative throw at the start and then returns to rest as continued tetanization maintains the new potential. On cessation of stimulation, if the nerve potential returns to its initial value, the galvanometer should give a positive deflection equal to the original negative one. It was observed from the start of these experiments, some years ago (see Gerard, 1927a), that the positive deflection was regularly greater than the negative one, often by more than 50 per cent. Control tests with constant or induced potentials through nerve or metal circuits have demonstrated the entire symmetry of the recording system—deflections in both directions are equal on closing or opening or reversing the direction of an applied potential. It seems to follow, then, that the fall of potential of the active electrode at the initiation of activity is less than the rise at cessation. Either there must be, following the initial sharp negativity, with continued stimulation a gradual further increase of negativity occurring too slowly to affect the galvanometer but leaving a greater negativity to rise from at the end of stimulation; or if the negative action potential is maintained constant there must be a positive after-effect on cessation.

A somewhat more direct demonstration of the positive deflection is obtained by short-circuiting the galvanometer during the tetanization so that it remains at rest. If the short circuit is then opened at once (by hand), after stopping tetanization, a positive deflection is obtained larger than the negative one previously determined. If an interval of about a second is allowed to elapse between the stop of tetanization and opening

the short circuit, little or no deflection is obtained. In all probability a marked and cumulative positive after-effect is being recorded. Such positive after-effects have long been known, and recently Gerard and Forbes (1928) have found them often present in the cat's peroneal. Amberson and Downing (1929) studying single impulses with a Downing galvanometer have offered entirely different evidence of their rather regular appearance in frog's nerves. This positive after-effect disappears considerably before the negative action potential does when a nerve is asphyxiated and returns after it on the readmission of oxygen. Table 3a.

It remains to discuss the changes in total action potential on repetition. Gerard (1927a) found the electric response of nerve to a given tetanization (280 shocks per second for 20 seconds) to become greater as the periods

TABLE 3a

Green frog sciatic. $T = 28°C$.

CONDITION	DEFLECTION AT				RATIO $\frac{\text{STOP}}{\text{START}}$	
	Start of tetanus		Stop of tetanus		Nerve I	Nerve II
	Nerve I	Nerve II	Nerve I	Nerve II		
Before N_2	−135	−120	+315	+240	2.3	2.0
25 minutes in N_2	−79	−66	+92	+69	1.2	1.0+
35 minutes in N_2	−23		+21		1.1	
40 minutes in N_2	−17	−7	+17	+6	1.0	0.9*
45 minutes in N_2	−5		+2		0.4*	
After O_2 4 minutes		−315		+400	1.4	1.3
7 minutes	−335		+485			
22 minutes	−205	−200	+350	+340	1.7	1.7

The fall of the ratio below 1.0 late in asphyxia indicates that fatigue is gradually reducing action potentials between the start and stop of the tetanization. A similar rapid fatigue was found by Fillie (1906) at the beginning of recovery following a period of asphyxia.

of tetanization were repeated at shorter intervals (10 minute intervals to 2 minute intervals). The thermal responses became less as the action potentials rose, and the rise of total action potential was interpreted as due to prolongation rather than increased magnitude of the negative potentials, and thus akin to fatigue. With prolonged tetanization the total action potentials fell, and in fact fell parallel with the fall in oxygen consumption per impulse under similar conditions (Gerard 1927b). This was shown by Gerard and Forbes (1928) (see also Forbes and Rice, 1929) to be due to decreased action potential per impulse and increased refractory period with fewer impulses per second. Decrease in positive and negative after-potentials resulted from slight repetition. Amberson and Downing (1929)

have found marked and long delayed positive and negative potentials which appear only on repetition of activity.

It is apparent from the foregoing that the total action potential is affected by several factors which change with the state of activity or equilibration of the nerve and it may therefore either increase or decrease as one or another predominates. The rise in total potential during equilibration may thus be correlated with increase of the delayed elements and the subsequent fall with decrease of the initial change (and secondarily of the delayed). These effects, as will be seen, are rather strikingly similar to those observed in the course of asphyxia. In the present experiments the usual definite rise of total action potential was noted when regular stimulation was begun after a period of rest in oxygen, but during and after asphyxia the situation was often reversed (see note to table 3a). It was also regularly observed that the rise was relatively greater with rapid tetanization than with slow, as would be anticipated if the effect is an equilibration to a new level of activity. (See table 2, data of Aug. 9.)

TABLE 4

CONDITION	RESPONSE TO STIMULATION AT		RATIO A/B	PER CENT OF FIBRES BLOCKED	PER CENT OF INITIALLY ACTIVE FIBRES BLOCKED
	A	B			
	mm.	*mm.*			
3 hours in O_2	43	48	0.90	10	
15 minutes in N_2	41	47	0.87	13	3
30 minutes in N_2	37	47	0.79	21	13
45 minutes in N_2	21	46	0.46	54	49
60 minutes in N_2	0	42	0.00	100	100

2. *Changes in and below an asphyxiated region during asphyxia.* It is convenient to consider later the changes at leads 1 and 2 above the region of asphyxia, and discuss here the changes at leads 3 and 4 in nitrogen and 5 and 6 below it. The graphs of several complete experiments are shown in figures IV, V, VI.

Results with the unbranched dog's peroneal may be analyzed first. The per cent of fibres blocked in passing through the nitrogen stretch is obtained by the fall in the ratio of A/B responses at lead 6 (see table 4). Thus in one experiment there were 3 per cent of the active fibres blocked after 15 minutes in nitrogen, 13 per cent in 30 minutes, 49 per cent in 40 minutes, and 100 per cent in one hour. At electrodes 3 or 4 at these times not more than the calculated percentage could be blocked but almost certainly less were. Failure of one fibre at any level will, of course, render it inactive distal to that point, and the longer the stretch of asphyxiating nerve above a given electrode the more the chance of such a critical point

being included. Electrode 6 (and 5) records impulses from A that have successfully traversed a 32 mm. stretch while 4 records impulses after 23 mm., and 3 after 9 mm. of travel in the nitrogen partition. Responses at

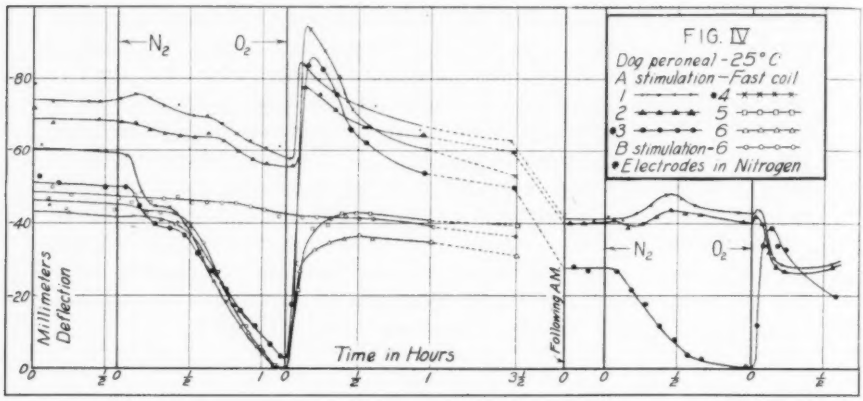

Fig. IV

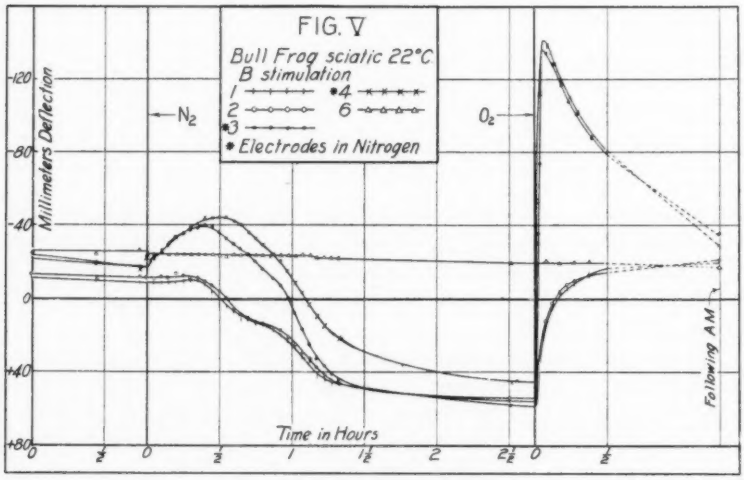

Fig. V

4 always fall faster and fail before those at 3, and those at 6 presumably do so faster than at 4. It is not safe to extrapolate back from 6 to 3 and 4, assuming a linear falling off with distance in nitrogen, since oxygen may

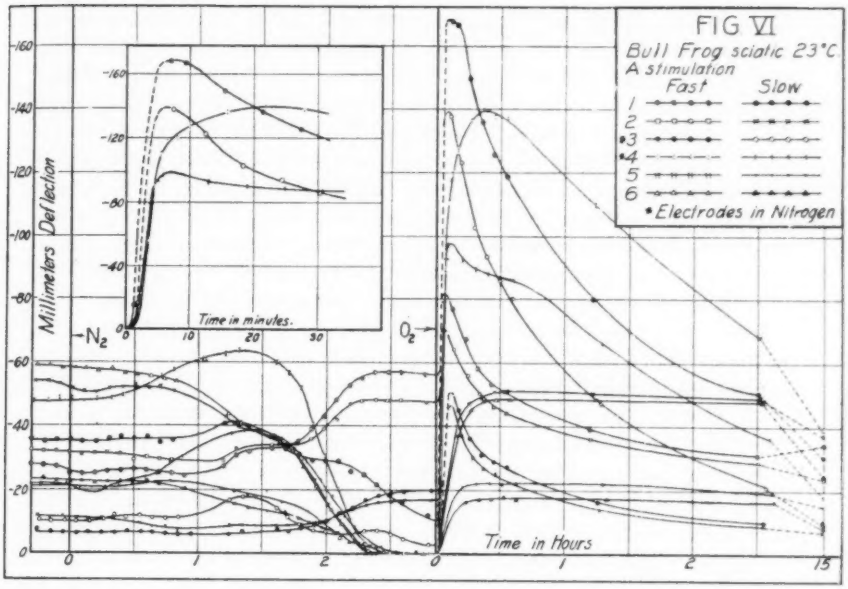

Fig. VI

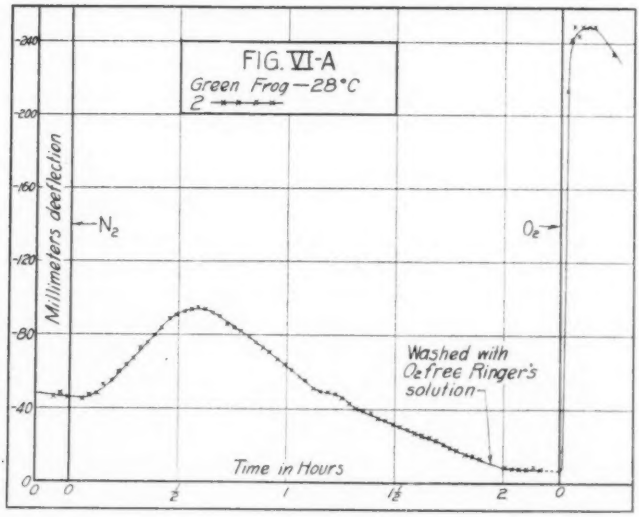

Fig. VIa

diffuse in from either end for an undetermined distance; but such a calculation would indicate that at ¾ hour, when half the fibres were blocked at 6, 35 per cent were blocked at 4, and 15 per cent at 3. Since oxygen diffusion from each side and the positions of electrodes 3 and 4 are symmetrical to the center of this stretch, though the value calculated for either one may be wrong (too high for 3 and too low for 4), their average must be essentially correct. At three quarters of an hour in nitrogen, then, the average of fibres blocked at 3 and 4 was 25 per cent. At this time, however, responses at 3 had fallen 58 per cent (from 50 mm. to 21 mm.) and at 4, 68 per cent (from 60 to 19–), with an average fall of 63 per cent. It appears, therefore, that 75 per cent of the fibres were able to yield at this time only 37 per cent of the original response, or that the action potential per impulse per fibre had been reduced to half.

In the last paragraph the possibility of decremental conduction has not been considered, nor the influence of prolongation of the refractory period discussed. The weight of recent evidence (Kato, 1926; Rice and Davis, 1928) disinclines one from invoking decremental conduction, though these results and other effects are subject to such an interpretation. An increased absolutely refractory phase, barring decrement, should decrease responses at lead 3 and beyond to the same degree rather than progressively more with more distal leads, as in the case of fibre block. This would modify the above calculations in the direction of a smaller decrease per fibre impulse, but even if the fall at 6 be assumed to be entirely due to increased refractory period, which would not account for 4 falling more rapidly than 3, the fibre impulse value in nitrogen would have fallen to less than three-fourths its value in oxygen. The further experimental analysis is made by comparing the responses to fast and slow tetanization.

When responses at 6 to fast tetanization fall sooner or further than to slow tetanization, an increase of the absolutely refractory period of the region in nitrogen is indicated. Increase of the relatively refractory period in the nitrogen stretch would, of course, not affect individual responses beyond it, so a fall in F/S at 6 directly follows a rise in absolutely refractory period in the asphyxiated region. In the exposed stretch, however, since the intensity of the propagated disturbance is decreased during the relatively refractory period, an increase in this period would serve to decrease the fiber-impulse value, and for fast tetanization more than for slow. Table 5 gives the results of an experiment with a dog's peroneal nerve. It will be seen that the average F/S at 3 and 4 fell from 2.4 to 1.5 during 20 minutes in nitrogen, while that at 5 and 6 fell from 2.7 to 1.5. The absolutely refractory period has increased sufficiently to block 40 per cent of the rapidly arriving impulses. Since the fall of the ratio at the electrodes in nitrogen is no greater than at those below, there is no evidence in this experiment for depression of the fiber-impulse value in nitrogen due

to impulses travelling in the less recovered part of the relatively refractory period. Yet the fibre impulse value at 4 had fallen to less than 50 per cent its initial value at 20 minutes.

The 40 per cent more rapid fall of total response to impulses at 3.3 σ intervals than to impulses at 11.2 σ cannot be taken to mean that the absolutely refractory period has increased to about 6 σ, and that none of the impulses at 11 σ intervals have been lost. The individual fibres are not all alike and evidence will be presented that they fail in nitrogen in groups, so the 40 per cent extra loss for fast stimulation is a purely statistical one due, possibly, to considerable prolongation of refractory period in some fibres and little in others. Even the responses to the slow rate of excitation may, therefore, have suffered some from prolonged refractory periods, though the effect would probably be but little.

An observation made several times in these experiments further substantiates the above discussion. The response at an electrode in the nitro-

TABLE 5

CONDITION	LEAD 3			LEAD 4			LEAD 5			LEAD 6		
	Fast	Slow	F/S	Fast	Slow	F/S	Fast	Slow	F/S	Fast	Slow	F/S
4 hours in O_2	24 5	10 5	2 3	23 5	9 5	2 4	27	11	2 5	22	7 5	2 9
10 minutes in N_2	15 5	7 0	2 3	11 0	5 0	2 2	25	9 5	2 6	19 5	7 0	2 8
15 minutes in N_2	10 5	5 5	1 9	5 0	3 0	1 7	13	7 5	1 9	11 5	5 5	2 1
20 minutes in N_2	5 0	3 0	1 7	1 5	1 0	(1 5)	3	2 0	1 5	2 5	1 5	1 7
30 minutes in N_2	1 0	0 5+		0	0		0	0		0	0	

June 21. Dog peroneal. T = 27.5°. Animal under ether 3 hours before nerve removed.

gen region to fast stimulation falls more rapidly than to slow and the curves may cross near the end of the period, so that the nerve gives no response to fast tetanization while still responding to slow. This Wedensky effect indicates that the oncoming impulse in normal nerve is just about able to excite the asphyxiated stretch when both are at "rest." At intervals of 11 σ the normal stretch will just complete its relatively refractory phase between impulses and a full sized disturbance follow each stimulus; at intervals of 3.3 σ the nerve, early in its relatively refractory phase, transmits subnormal impulses which are insufficient to excite the asphyxiated portion with its increased threshold. A further factor would operate when an increased interval between stimuli allows the oncoming impulses in the normal nerve to attain the threshold intensity for exciting the asphyxiated stretch. This latter has a prolonged total refractory period, or prolonged rise of threshold after conduction, so that after one response its threshold will still be high when the next one or several impulses reach

it from the normal stretch, and they will be blocked. The fact mentioned above, that the F/S ratio below the region of asphyxia falls as much as in it, indicates that this complete blockage of impulses as they impinge on a high threshold region is the main result of prolongation of the absolutely and relatively refractory periods. Figure VII illustrates these points.[3]

It may be noted that by study of the conditions of block of a nerve it should be possible to evaluate quantitatively the separate factors, for conduction, of 1, the exciting strength of an active region upon an adjacent resting one, and 2, the excitability of the resting region. We hope to pursue this matter. The mechanism of cold block is probably also very similar to that here outlined; and Heinbecker's (1929) finding that the refractory period of each fibre type rises to about the same value before failure occurs is also in harmony with these considerations.

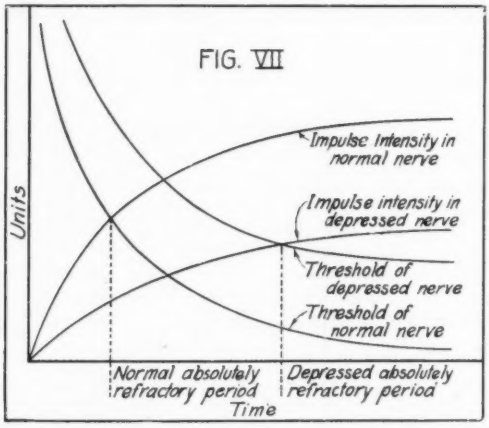

Fig. VII

To sum up the results so far, the data indicate that during asphyxia the intensity of a single impulse for a single fibre may be reduced to half, that the refractory period increases enough to cut out an average of one-third of the impulses arriving at 3.3 σ intervals, and that the ultimate complete failure is due to blocking of all the fibres. Different fibres block at different times, probably each one as the lowered exciting power of its own re-

[3] It may be well to point out that the tests used for absolute refractoriness have depended on a response at a distance from the point stimulated, and therefore involve conduction. The absolutely refractory period, then, probably is not a measure of the time during which a nerve region is inexcitable but of the time during which activity of this region is unable to excite adjacent ones; i.e., the time of blocked conduction.

TABLE 6

Bull frog sciatic, T° = 23°, condenser circuit. March 25

CONDITION	LEAD																	
	1			2			3			4			5			6		
	F	S	F/S	F	S	F/S	F	S	F/S	F	S	F/S	F	S	F/S	F	S	F/S
2 hours rest n O_2	27	7+	3.7	33−	10	3.3	35+	11−	3.3	48	22	2.2	54	22	2.5	59	23−	2.6−
N_2 in middle partition																		
45 minutes	26	7	3.7	30−	8	3.7	34	11	3.1	57	27	2.1	52	22	2.4	55	22	2.5
80 minutes	32	7	4.6	33	9	3.7	40*	17*	2.4	64*	39*	1.6+	39	19	2.1−	40	16	2.5
120 minutes	44	10	4.4	40	11	3.6	29	8	3.6	30	21	1.4	16	9	1.8	16	8	2.0
150 minutes	57	19	3.0	49	16	3.1	18	6	3.0	0	1	0.0	0	1	0.0	0	1	0.0
At 180 minutes O_2 admitted																		
6 minutes	78*	53 *	1.5	65*	48*	1.4	168*	138*	1.2	118 (140*) 22 min.	98	1.2 (1.4)	36	18	2.0	32	12	2.7−
60 minutes	41	19	2.2	37	16	2.3	89	57	1.6−	117	73	1.6	51	22	2.3	48	17	2.8
150 minutes	31	10	3.1	29	9	2.3	50	21	2.4	57	28	2.0	49	20	2.5	48	16	3.0
Values as fractions of initial																		
Maximum in N_2	2.1	2.6		1.5	1.4		1.15	1.6		1.35	1.8							
2 hours in N_2	1.6	1.4		1.2	0.95		0.8	0.75		0.61	0.95		0.3	0.4		0.3	0.35	
Maximum in O_2	2.9	7.2		2.0	4.8		4.8	13.0		2.9	4.5		0.95	1.0		0.8	0.75	
2½ hours in O_2	1.15	1.4		0.9	0.8		1.4	2.0		1.2	1.7		0.9	0.9		0.8	0.7	

* Indicates a maximum value.

sponse falls below its increased threshold. The relation of oxygen exclusion to these changes will be considered later.

The experiments with green-frog and bull-frog nerve have yielded similar results. Representative data are included in table 6. In most cases, responses at the lower electrode in nitrogen did not fall lower than those at the electrode below nitrogen but they approached zero together. In several instances, however, the response in nitrogen was depressed to as low as half that of a more distal electrode in oxygen. A lowered fibre-impulse response is, therefore, present in these nerves though to a less striking degree than in the case of the dog nerves.

In two experiments it was noted near the end of an asphyxial period that electrodes five and six still gave small negative responses while responses at four had passed through zero and were weakly positive. A few minutes later all responses were zero. Current escape appears to have been excluded, so the positive effect must have represented a positive action wave at four or a negative change at seven, leading from the "dead" end. The latter is probably the case. It has been pointed out that with activity of a nerve the negative change at the crushed end is rarely absent, actually it may be two-thirds as great as along the intact side (see later). If impulses in the fibres still active in nitrogen were depressed to less than half their normal intensity and returned to their usual value on entering the oxygen supplied stretch beyond, the negativity at lead seven would be greater than at lead four. Four would then appear to become positive until complete block brought all responses to zero.

In all experiments, the F/S ratio fell markedly before the end of asphyxia For the three best, for example, the average ratio at the electrodes in nitrogen fell from 3.1 to 1.7 and at the distal electrodes in oxygen from 3.1 to 2.0.

The conditions toward the end of an asphyxial period have been discussed so far. In the earlier stages the situation is quite different. The responses in nitrogen show a marked increase at a time when the electrodes in oxygen below are still constant. The fibre-impulse total action potential in nitrogen has therefore increased in amplitude, duration or both. An increase in amplitude of potential, especially if due to changes of electrical impedance of the nerve, should involve the fast and slow responses proportionally and leave the F/S ratio unchanged. A prolongation of negative potential or decrease in positive after-effect should be more effective for the slow than the fast and cause the ratio to fall. The fall in F/S is quite marked during the rise of total responses and may reach an even lower value than later during asphyxia. This is evidence of a marked prolongation, or shift in the negative direction, of the action potential in nitrogen, though impedance changes may also be present. This is in harmony with the earlier finding (Gerard, 1927) that the total action

potential of a given nerve falls much less rapidly in nitrogen than does its heat production. Heinbecker (1929), using the cathode-ray oscillograph, finds no change in the shape of the action potential during asphyxia. He was not recording, however, delayed potential changes, where the greatest modification probably occurred. A similar discrepancy between the findings with the Braun tube (Heinbecker) and with a slow galvanometer (Amberson and Downing, 1929; Necheles and Gerard, 1929) in the case of carbon dioxide action on nerve also seems to depend on the delayed potential changes. The changed form of the electric disturbance in nitrogen makes it impossible to make accurate comparisons of refractory period effect in and below nitrogen. Since, however, any impulse emerging from the asphyxiated stretch must resume its normal form, the F/S values at leads 5 and 6 do give valid information as to refractoriness, as already discussed.

This early rise in nitrogen is usually but not invariably present. It is nearly always seen in the bull-frog nerve, often absent in the green-frog. When failure progresses rapidly it may be represented by only one or two readings. In previous experiments on R. esculenta (Gerard, 1927a) a prompt rise in nitrogen was attributed to a changed rate of stimulation, since it disappeared when stimulation was repeated at constant intervals. In this series the less abrupt rise has appeared despite this precaution.

The responses in nitrogen showed an average increase at their maximum to 1.55 times the initial value. This was reached soon after responses below the nitrogen stretch had begun to fall. That is, responses at 5 and 6 began to decrease just before those at 4 did, and those at 3 began to fall a little later. The form of these curves gives the impression that the rise of response due to prolongation of potentials is continuing but is cut into by rapidly appearing fibre block. Later, apparently, decreased amplitude of each action potential more than offsets its prolongation and the area of the potential-time curve is again diminished.

After responses at 4 began to fall they sometimes dropped very rapidly and largely by fibre block, as if some factor necessary to conduction reached a critical value. Usually, however, they fell more slowly and showed definite waves of decrease (fig. IV). Identical waves were seen in the fall of responses at 5 and 6 and less markedly at 3, so that fibres were apparently failing in groups. There were commonly two waves, sometimes three, probably representing a differential susceptibility of large and small fibres to asphyxia. Heinbecker (1929) similarly found that asphyxia blocked the fibres in groups, the smaller fibres failing before the larger.

The responses at various electrodes to stimulation at *B*, have been in harmony with the results for stimulation at *A*. For the latter, responses at 4 fall more rapidly during asphyxia than those at 3; for stimulation at *B* responses at 3 fall before those at 4. In either case responses at the elec-

trode reached by the impulses over a long asphyxiated stretch fall faster than at the nearer electrode. Any possible pre-existing polarity of the nerve, effect of branches, or distance between active and injured leads is excluded as a factor; and the effect must be due to fibre block at different points (or decrement), as previously discussed.

In all cases with *B* stimulation, as asphyxia proceeded, the responses at 4 and 3 in nitrogen, and 2 and 1 beyond it, fell to zero and continued to "fall" until strongly positive. This was not due to the Fleischl effect, since reversing the coil leads left the deflections in the positive direction. No local current spread was involved, the positive throw was equally great at electrodes 30 and 70 mm. away from the stimulated region. Arising at *B*, impulses pass in one direction to 6 and 7 (the crushed end) and in the other through the region of asphyxia to 2 and 1. A reduced response at 7 is regularly present and the normally greater response at 1 or 2 leads to negative action potentials from the two usual leads. But the progressive asphyxial block in no way affects the impulses travelling from *B* to 7, while decreasing to zero the impulses reaching 2 or 1. The crushed end thus becomes negative to the normal nerve beyond an asphyxiated stretch. The maximum positive deflection obtained measures the activity at 7; the total change from minus to plus, the normal activity at 2 or 1. This evidence, as that given elsewhere, shows that after some hours' standing, activity at 7 may be over two-thirds as great as at an uninjured point.

It has been assumed in the discussion so far that asphyxial block renders the responses fully monophasic. Even if the block by asphyxia did not completely stabilize the potential at electrodes beyond it, their potential changes would be less than previously appeared at the crushed end; the latter having undergone recovery changes for some hours. Reasons for the presence of activity beyond a crush have been indicated by Bishop, Erlanger and Gasser (1926). Essentially, the nerve beyond the crush serves as an inert lead from some point at the crush which has a potential intermediate between the positive surface and the negative core. Fall in potential of the surface must give a proportionate fall of all points along the potential gradient between surface and core, and hence a fall at the lead involved.

The conditions at a crush may be analyzed somewhat further. Figure VIIIa represents schematically the electrical situation along a stretch of nerve near a crush. Points on the surface of the membrane separated from each other by the low resistance, r, are maintained at a potential above the core by batteries, giving potential e, in parallel. There is a high resistance leak, R, across the membrane. At the point of injury to the membrane this transverse resistance is greatly lowered to R_1. It is readily shown that current will then flow from points away from the injury along the surface

to the injured point and back along the core, producing a potential drop along the nerve. The current flowing from any point will be less as its distance from the injured point, and so the longitudinal resistance, increases. The core-surface potential must, then, begin to decrease at some distance from the point of injury and become steadily less as the injured

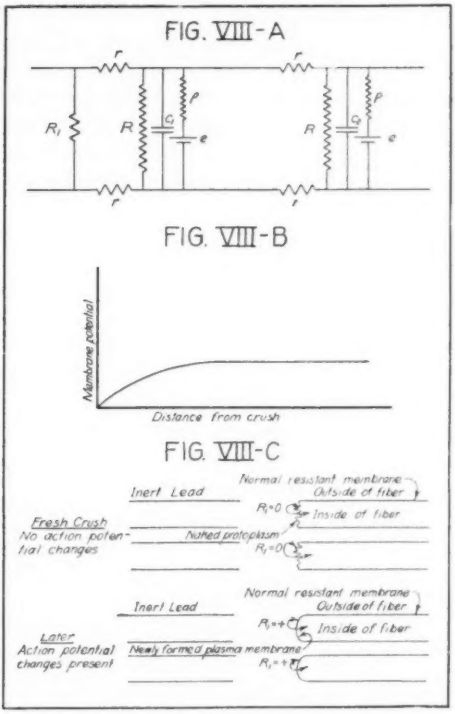

Fig. VIIIa. R = high resistance leak across intact membrane. R_1 = low resistance short across injured membrane. r = moderate longitudinal resistance. c_1, c_2 = polarized membrane acting as capacity. e = constant source of potential charging membrane through resistance. When $R_1 = R$, the potentials across the membrane at c_1 and c_2 are equal. When $R_1 < R$, the potential across c_1 is less than across c_2.

region is approached. If R_1 is zero then this potential must fall to zero at the crush (fig. VIIIb). The closer R_1 approaches R, the less the potential drop across it. When R_1 is not zero the conditions are those considered by Bishop, Erlanger and Gasser; that is, the nerve beyond the crush serves as a simple lead from some point at a potential, not zero, which is a constant

fraction of e. Since the membrane potential, e, falls during activity, the lead from the crush also shows a smaller fall in potential.

As pointed out, the action potentials at the injured end increase for some time after a crush is made and are again reduced by a fresh crush. At the

TABLE 7a

Green frog sciatic—28°C.

CONDITION	RESPONSE, LEADS 3 AND 4
10 minutes after dissection—no crush	77
Crushed 3 mm. above lead 4	
1 minute	133
8 minutes	116
10 minutes	112
5 hours	75
Crushed distal to first crush	
1 minute	76
Recrushed 3 mm. proximal to first crush	
30 seconds	165
90 seconds	158
4 minutes	153
9 minutes	146
2 hours	120

TABLE 7b

Bull frog sciatic

CONDITION	RESPONSE FROM LEAD 6 AND									
	1		2		3		4		5	
	Deflection	Change	Deflection	Change	Deflection	Change	Deflection	Change	Deflection	Change
No crush										
20 minutes after dissection	26		28		23		4		3	
3 hours after dissection	11	−15	7	−21	3	−20	3	−1	0	−3
Crushed 3 mm. above lead 6										
1 minute	130	+119	130	+123	112	+109	130	+127	81	+81
2 hours	107	−23	116	−14	100	−12	107	−23	68	−13
Recrushed proximal to first crush										
1 minute	122	+15	131	+15	115	+15	126	+19	73	+5

moment of injury the cell membrane is destroyed, which reduces the transverse resistance, R_1, and the action potentials at the crush, possibly to a practical zero. Later the naked protoplasm has formed some "surface-interior" membrane (see Chambers, 1924); a definite transverse resistance is reëstablished, and with it a potential change during activity (fig. VIIIc).

The nerve membrane potential falls markedly during oxygen deprivation (see later), probably due to lowering of the transverse resistance and to decrease of the potential developing reactions. Either factor would lead to a decline of potential on passing from normal to asphyxiated nerve. Whether the potential in the nitrogen stretch falls to zero is uncertain, but it apparently does fall to lower values than at an old crush.

It is worth noting that the above considerations account for the coexistence of depressed responses and heightened irritability near an injured region and as a result of catelectrotonus (Bishop and Erlanger, 1926), since a partially depolarized membrane would require less current to complete its depolarization and give less potential change on depolarization than a normally polarized one—other factors remaining unchanged.

TABLE 8

NERVE	DATE	TEMPERATURE	UPPER ELECTRODE IN N_2. TIME TO		LOWER ELECTRODE IN N_2. TIME TO			ELECTRODE BELOW N_2. TIME TO	
			Maximum	½	Maximum	½	Block	Start of fall	½
		°C.	*minutes*	*minutes*	*minutes*	*minutes*	*minutes*	*minutes*	*minutes*
Dog		25		40		35	67		45
Dog		28	3	14	3	10	24	5?	16
Bull frog	December 6	22	108	215	87	235	>300	72	108
Bull frog	April 20	22	78	320	68	156	183	54	113
Bull frog	March 25	23	78	150	80	123	150	48	110
Bull frog	April 26	26	56	96	51	95	118	45	83
Green frog	December 28	24		67		45	150		54
Green frog	August 11	26		55		22	40		
Green frog	August 7	28	20			20	30		
Green frog	July 14	27		34		28	35		20

3. Time course in N_2. The time required for a nerve to fail in N_2 varies with the species of nerve and the temperature during the asphyxia (table 8), also with the amount of activity. For each kind of nerve asphyxia proceeds more rapidly at higher temperatures and, though the data do not justify the calculation of a temperature coefficient, it would appear to be over 2. At a given temperature dog nerve asphyxiates most rapidly, green frog nerve definitely slower, and bull frog nerve considerably more slowly. The ability of a nerve to withstand oxygen lack depends in large part on the amount of oxidizing reserve it possesses and the rate at which this is used up. A higher temperature causes a more rapid utilization (the temperature coefficient of oxygen consumption is over 2.0) and hence more rapid asphyxia. The sciatic of a rabbit consumes oxygen almost twice as rapidly as a grass frog sciatic at the same temperature (Gerard, unpublished), and presumably the dog nerve would resemble the rabbit's.

The bull frog sciatic consumes only half as much oxygen as the green frog nerve (Gerard, unpublished). The above observations are, therefore, what would be expected on the basis of differences in the rate of utilizing a reserve; though, of course, differences in the initial reserve may exist. The present data are not sufficient to confirm or refute Gottschalk's (1919) claim that nerves from cooled frogs have a greater oxygen store than from warmed ones, but his results are equally susceptible to interpretation on the basis of differences in metabolic rate.

It may also be pointed out that, given a sufficient time for recovery in oxygen following a complete asphyxiation, a second asphyxiation progresses hardly more rapidly than the first. After short periods of recovery in oxygen, the second asphyxiation was found by Fillie (1908) (and more fully studied by Gottschalk, 1914, 1919) to progress more rapidly. Since a nerve takes up an excess of oxygen for an hour or more after an asphyxial period (Gerard, 1927b) it appears that the oxidizing reserve is entirely exhausted during complete asphyxia, is slowly regenerated in oxygen and when fully restored permits the nerve to withstand the same duration of oxygen lack as at first. The exhaustion of such a reserve must, then, be the critical factor in asphyxia. The higher the metabolic rate and the lower the reserve of each fibre or group the sooner does it asphyxiate. If the store of oxidizer is a function of the volume of a fibre, and the energy used in conduction a function of its surface, the smaller fibres should asphyxiate before the larger ones, as Heinbecker (1929) finds to be the case.

4. Changes in and below an asphyxiated stretch following readmission of oxygen. At the end of a sufficiently long period of asphyxia, responses to stimulation at the electrodes in and below nitrogen have fallen to zero. On now admitting oxygen to the nitrogen partition, there was a rapid and spectacular return of action-potentials at these electrodes to high values. Activity appeared in about a minute at all electrodes. At those leading from the nitrogen stretch, responses increased to a maximum in 5 to 10 minutes, usually, and then declined, rapidly at first and then more slowly, along a roughly exponential curve. With either *A* or *B* stimulation, that electrode in the nitrogen partition which was more distal from the stimulus showed a later maximum than the other and a longer persistence of the increased responses. The maximum responses obtained were not merely to the height of those before asphyxia but were very much greater, even six times as great for the fast tetanization and twice this figure for slow! Such a great response was obtainable only at the "peak" of the curve, lasting a few seconds. The subsequent falling off did not bring the responses at the more distal electrode back to "normal" values for as long as five hours or more. In a few instances, the maximum responses did not appear in the time mentioned but were reached in half an hour or more.

Responses at electrodes 5 and 6, beyond the asphyxial block, returned

usually to some maximum value which was then maintained. The maximum reached was usually somewhat below the values at these electrodes before asphyxiation. In several cases, however, where the initial decrease in responses of the freshly mounted nerve had been largely completed before asphyxia was begun, the action potentials returned to these original values after complete asphyxia, and practically maintained them over night. In two cases at least the nerves were mounted after dissection without having been moistened with Ringer's solution, except for a small drop at each electrode. They were asphyxiated and showed good recovery without application of Ringer's solution, and were moist and active the next morning. Further, nerves have been several times asphyxiated in nitrogen and at once washed in oxygen-free Ringer's solution with no return of activity. See figure VIA.

It has been often reported (Fillie, 1908; Gottschalk, 1919; Cooper, 1923) that, following asphyxia, partial recovery follows admission of oxygen, or oxygen-free Ringer, but full recovery is possible only with both. The inference has been drawn that in asphyxia part of the failure is due to the accumulation of diffusible but not oxidizable break-down products. It has been shown (Gerard and Meyerhof, 1927) however, that lactic acid accumulation does not interfere with its further formation and that nerves in gas or in Ringer's solution behave alike during asphyxia.

Nerve does form lactic acid under asphyxial conditions as a product of its *resting* metabolism, and it is not able subsequently to oxidize a significant amount of it (Gerard and Meyerhof, 1927; Holmes and Gerard, 1929). Heinbecker (1929) finds that a high concentration of lactic acid applied to a nerve depresses its activity. This may be due to acidity, which develops after the nerve buffers are exhausted. In this case smaller amounts would have little effect. Under the usual conditions of asphyxia there is not more than 20 to 30 mgm. per cent of lactic acid formed during the time to complete block, whereas Heinbecker's experiments were performed with concentrations of 250 mgm. per cent. It is, of course, impossible to deny any importance to the accumulation of unoxidized metabolites in the course of nerve asphyxiation, but to date the direct interference with oxidations serves adequately to account for the changes observed when nerve is subjected to oxygen lack.

The return of responses at leads 5 and 6 was seldom complete before a quarter of an hour, occasionally not before three-quarters. Probably lower temperature and relatively longer deprivation of oxygen slowed recovery, though no detailed analysis of these data has been made. The peak of the responses at 3 or 4 was reached regularly many minutes before 5 and 6 had come to maximum responses; that is, when many fibre-impulses were still missing at 4 the responses were higher than later when they were present. In the experiment of May 21st, previously analyzed,

the maximum responses at 3 and 4 were obtained at about 5 minutes, when those at 5 were less than 70 per cent of the value reached 10 minutes later.

The gradual return at electrode 5 seems to be a measure, primarily, of the reappearance of activity in successive fibres. A variation in time of recovery for different fibres is entirely comparable to the variation in time of failure; and in fact most of the changes following admission of oxygen mirror, on an accelerated time scale, the changes during asphyxia. This is true for the F/S ratios at leads 5 and 6. These were seen to fall steadily in nitrogen; on admission of oxygen they showed a similar but more rapid rise to, often, the original value (table 6). In cases where a Wedensky effect had appeared at the end of asphyxia and F/S had fallen to less than 1.0, the same condition was seen at the start of recovery and the curves at lead 5 for fast and slow tetanization crossed. This is evidence, then, for the gradual (though relatively rapid) shortening of the absolutely refractory period, and the successive return to function of the various blocked fibres when oxygen is made available.

The responses at leads 3 and 4, reaching such high values even when some fibres are still inactive, show that the time-potential value for a single fibre-impulse has increased enormously. Again, the comparison of fast and slow tetanization permits analysis into increased amplitude and duration. The F/S ratio at the peak in oxygen has fallen to values considerably lower than those recorded during asphyxia (of course toward the end of asphyxia no ratios are obtainable), and then rapidly rises to values close to the original ones before asphyxia. The peak responses for slow tetanization may be almost as great as those for fast; and the conclusion follows that the action potentials are greatly prolonged. An increased amplitude may also be present, probably as a result of breakdown during asphyxia of the transverse resistances in the path of the current from axones to leads, but this cannot be the sole effect since it would not explain a changed F/S ratio.

The later peak at the more distal nitrogen electrode than at the nearer one in the present results is easily understood in terms of fibre recovery. Just as block appears earlier at 4 than 3 because of the longer exposed stretch, so recovery of fibres would be slightly later. It is more difficult to understand why the fall after the peak is often much slower at 4 than 3. The peaks for slow tetanization are similarly reached before those for fast because early during the recovery in oxygen the return of fibre-impulses, as the refractory period becomes less than the stimulus interval, is relatively greater for less frequent stimuli.

It may be well, before proceeding, to consider the factors influencing the total action potential as recorded. The *amplitude* depends on the following. *1. The fraction of total fibres active and the number of impulses carried by each.* These do not affect the fibre-impulse values. *2. The physical*

conditions of the circuit. A decrease in the transverse sheath resistance between the site of the axone potentials and the leads, or an increase of the longitudinal resistance (tissue fluid or Ringer solution) between adjacent active and inactive points on the axones would cause a greater fraction of the potential developed to reach the galvanometer. Such changes are also unrelated to true functional modification of the active fibres. But the sheath resistance probably depends largely on living polarizable surfaces which might be rendered more permeable during asphyxia, in much the same way as the nerve fibres proper. *3. The physical condition of the axone membranes.* A decreased transverse resistance allows a greater leakage of current and so tends to lower the resting membrane potential and, secondarily, the action potential. It might also, however, depending on the exact site and means of production of the action potential, tend to increase the recorded change in a manner similar to that considered in (2). *4. The chemical condition of the axone (membrane?).* The basic reactions supplying the energy and conditions (special substances?) for maintaining the membrane potential may be decreased, leading directly to a fall in resting and active potentials. It is of course arbitrary, though convenient, to distinguish (3) and (4) since a complex physico-chemical situation is at the basis of both. Changes in the delayed potentials are subject to these factors but are more conveniently considered in the next group.

The *duration* of the action potential depends also on several factors. *1. The rising phase of the main potential wave.* This appears to be of an explosive character and little influenced by the metabolic condition of the nerve. *2. The falling phase of the main wave.* This has a higher temperature coefficient than the rise (Adrian, 1921, Bishop and Erlanger, 1926) and has been reasonably correlated with the early stage of recovery. Adrian (1921) found the duration of the fall of potential and of the absolutely refractory period to vary approximately together with changes in temperature. Bishop and Erlanger (1926) found the absolutely refractory period prolonged by cathodal polarization and the time of fall of potential shortened. The slopes of their potential curves show, however, that the *rate* of fall of potential in cathodal polarization is, if anything, slowed. They end sooner than normally because the fall begins from a lower level. Presumably the absolutely refractory period represents the time required for a certain *amount* of recovery (substances formed, membrane rebuilt, etc.), as indicated by the findings of Gerard, Hill and Zottermann (1927). If the falling potential is a measure of this same recovery, which is zero at the end of activity, then the refractory period would not vary as the total duration of the falling potential but as the time required for a definite amount of fall. Choosing some arbitrary amount of fall (10 mm.) it appears from Bishop and Erlanger's figures that the time from the start of the response to this point is actually increased by cathodal polarization.

In the case of asphyxia, however, Heinbecker (1929) reported no change of duration of the main axone potential wave despite a prolonged refractory period. If this is correct it would indicate that the falling action potential does not measure recovery in the same sense that the refractory period does. *3. The negative after-effect (remainder, delayed potential).* This is presumably related to the delayed heat production and oxygen consumption associated with an oxidative recovery. Its magnitude and duration might be expected to show great variations with the functional state of the fibres, as will be discussed. *4. The positive after-effect.* This also appears to be closely related to metabolic changes in the nerve. It is tempting to regard it as an "over repair" phenomenon, especially as it disappears early in asphyxia, but its real nature is little understood. It

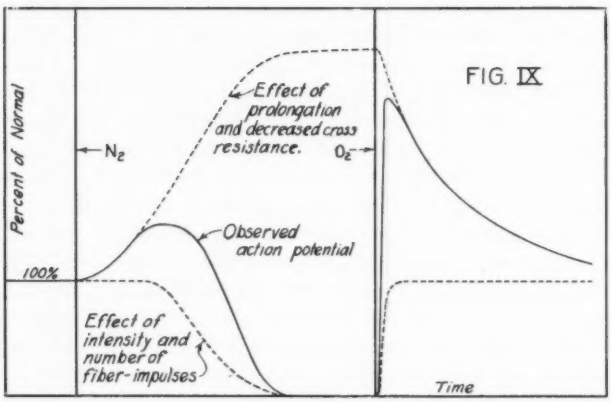

Fig. IX

can hardly represent the negative after effect at the injured end, for then it would increase rather than disappear as the side is asphyxiated. *5. The degree of summation of the delayed potentials of successive impulses.* This is certainly not complete but may vary with the condition of the nerve.

During asphyxiation the region of nerve involved exhibits first an increased response—associated with lengthening of the action potential and probably increased amplitude due to lowered transverse resistance, and then a fall—associated with lowered potentials due to depressed chemical reactions and greater membrane "leakage," lengthened refractory period, fibre failure and ultimate complete block. In recovery there appears first a steep rise as fibres again become active and responses are greater than at any previous time, due in large part to great lengthening of the action potentials. Following this there is a fall of response despite continued

and improving fibre activity, due to the return to more normal durations of the action potentials.

The picture is clearly that of two groups of antagonistic influences. During oxygen lack the return to resting potential after activity is progressively delayed, tending to give ever greater responses. This delay is rapidly reversed when oxygen becomes available and responses decrease to their original level. Were it not for fibre block (and feeble potentials), the curve for responses during asphyxia and recovery would always be above normal, reaching or maintaining its maximum value at the time of reoxygenation, and this maximum would be considerably greater even than that actually reached during recovery (see fig. IX). Fibre block and low potentials do supervene, however, later during asphyxia than the appearance of delayed potentials, and rapidly abolish all response. During recovery, block is relieved more rapidly than the changes causing delayed potentials—whence the sharp peak of responses. Both prolonged action potential and lowered action potential (increased refractory period and block) are expressions of a delay in one or more chemical changes necessary to restore the nerve to its resting activable state following an activation.

Assuming the delayed potentials to correspond to the early part of the delayed recovery process, their behavior is easily understood if the amount of potential is an index of the recovery still to be completed. In fresh nerve they are absent but they appear on activity as a state of equilibration develops. At the same time heat production and oxygen consumption fall. They appear during asphyxia when heat production is decreased and probably prolonged (Gerard, 1927a). They appear under treatment by CO_2 which seems to depress recovery processes. Interference, by any of these means, with the membrane restoration following activity should lead to the persistence of lower (negative) potentials. Levin (1927) likewise related delayed potentials to incomplete recovery in the case of crab nerve though he assumed a lactic acid mechanism.

According to the scheme suggested previously (Gerard, 1927c) oxygen lack causes gradual depletion of the oxidizing reserve (probably intermediate substances in the chain of normal oxidation reactions). This progressively shows and diminishes the oxidative recovery reaction (= prolonged potentials) and decreases one or more of its necessary end products. Decrease of these slows the immediate recovery processes (= prolonged refractory period) and finally diminishes and stops the initial reaction of conduction (= decreased initial potential and block). On readmission of oxygen the reactions again get under way, but since the delayed one is relatively slow some minutes are required before recovery of the immediate ones is sufficient for conduction to reappear. Also, since an hour or more is required for the replenishment of the oxygen reserve, the delayed potential persists for some time.

The picture here sketched in is obviously too simple to represent the full effects of asphyxia on nerve activity, but it may serve as a first approximation. It is obvious, if this analysis is correct, that the peak in oxygen depends on the separation in time of two antagonistic changes. If disappearance of delay were more rapid than or simultaneous with disappearance of block, then no peak would appear, and responses at 3 and 4 would return along the same smooth curve as at 5 and 6. Altered time relations might be possible if the two effects did not depend on the same chemical reaction, and if obtained would constitute evidence of such independence. Change in temperature (reactions with different temperature coefficients) in duration of asphyxia, etc., might thus modify the appearance of the peak. So far no direct experiments have been made to test the effect of temperature. In the work reported previously (Gerard 1927a) the return of heat production and of action potentials following asphyxia gave no evidence of a peak in any experiment. The main differences in conditions were 1, the nerves were kept at an average temperature about 10°C. lower; 2, the duration of the asphyxial period was longer (but not longer in relation to the lower temperature); and 3, the entire nerve, including the stimulated region, was subjected to asphyxia. Since experiments were not completed for many hours, even a day, after the crush, responses must have been largely diphasic; and as the changes due to asphyxia occurred under both leads the effect of prolongation was largely cancelled. This, rather than the temperature difference, is probably the reason that the peak did not appear.

5. Changes at electrodes proximal to an experimental region. A priori, one would hardly anticipate any change in responses led from normal nerve when the region subjected to experimental modification lies *beyond* it; that is, when impulses arise and travel in unaltered nerve up to the active lead. So generally has this been assumed that such proximal control leads have regularly been dispensed with. In the present series, one was included to permit (by comparison with a lead below the experimental stretch) an estimate of blocked fibre-impulses, but it was rapidly found to be useless for this purpose since responses from it suffered striking changes during the development of asphyxia below. A second electrode was added above the nitrogen stretch to secure further evidence of the nature of these changes.

In all cases leads 1 and 2 varied together, and if there was any difference in the magnitude of the change it was greater at 1 than 2 (table 9). The typical picture, seen in most cases, is that of figure 6 (see also fig. XII). One and 2 show no change while 5 and 6 remain constant, even though 3 and 4 are rising. As 5 begins to fall, 1 and 2 start to rise; and in general the curves at these two leads are fairly the inverse of that at 5. This is true, in the best experiments, even to a duplication of the waves in the fall of 5 with waves in the rise of 1 and 2.

The interpretation of this particular change seems clear cut. The partial activity at "killed" lead 7 normally diminishes potentials led from 1 and 2. As fibres block in nitrogen this activity is lessened or abolished and the recorded potentials at 2 increase. This increase would then start as impulses reaching 7, and therefore 5, begin to decrease, and should mirror

TABLE 9

NERVE	TIME IN N_2	UPPER ELECTRODE (1)		LOWER ELECTRODE (2)		TIME IN O_2	UPPER ELECTRODE (1)		LOWER ELECTRODE (2)	
		Response (Fast) / Initial	F/S	Response (Fast) / Initial	F/S		Response (Fast) / Initial	F/S	Response (Fast) / Initial	F/S
	hours									
Bull frog	start	1.0	3.7	1.0	3.0	6 minutes	2.9	1.5	2.0	1.4
	1⅓	1.2	4.6	1.1	3.7	2½ hours	1.1	3.1	0.9	3.2
	2½	2.1	3.0	1.5	3.1					
Bull frog	5	4.0		4.4		15 minutes	0.8		0.4	
						45 minutes	1.0		0.9	
Bull frog	2	1.4		1.5						
	4	1.3		1.4						
Dog	1	0.8		0.8		8 minutes	1.15		1.13	
Dog	start	1.0	2.0	1.0	2.3	5 minutes	1.45	1.5	1.35	1.5
	½	0.9	2.1	0.9	2.6	1 hour	0.9+	2.2	0.9−	2.6
Green frog	start	1.0	1.8			10 minutes	1.1	1.3		
	½	0.4	3.5			5 hours	0.7			
	¾	0.1	1.4							
Green frog	start	1.0	1.5			5 minutes	2.4	1.4		
	1	1.5	2.1							
Green frog	start	1.0	5.0			5 minutes	1.3	2.1		
	1	0.8	8.5			7 hours	0.7	6.5		
	2½	1.0	4.5							

the changes shown. In one experiment, action potentials at 1 rose from 34 mm. before asphyxia to 134 mm. when asphyxial block was complete, the largest rise observed. After a night in oxygen the action potential was 41 mm. A fresh crush was then made above the old one and action potentials at once increased to 134 mm., over three times. Activity at 7 must have been at least ⅔ that at 1. (See tables 7a and b.)

Further, the F/S ratio at 1 and 2 *rises* as the curves rise and later falls to its initial value or somewhat lower (table 9); while at all other electrodes F/S falls from the start. The more complete monophasicness of response will also account for the changed ratio, since responses at 5 (and 7) fall off more rapidly for fast than for slow stimulation, and a temporary increase in F/S at 1 and 2 must result. Ultimately both are completely abolished and the ratio returns towards its original value.

Unfortunately for the simplicity of this interpretation, it does not account for other effects. In several experiments (especially with green frog nerves) the proximal electrode responses fell along with those in nitrogen, though less fully, probably because of proximity to the asphyxiated stretch; and in two cases the responses fell and then rose above the original values or rose and then partly fell, although the lower electrodes were responding in the usual manner.

A more serious difficulty is met in understanding the changes at 1 and 2 on readmission of oxygen to the asphyxiated stretch (figs. IV, VI, table 9). Responses at 1 and 2, as at 3 and 4, show definite "peaks" within a few minutes after the admission of oxygen, though these rises are much less than within the experimental stretch. The peaks at 1 and 2 have been absent or small when, during a long period of asphyxia, responses at 1 and 2 have been maintained at high values. Whether the peaks were large or small, there was always a very sharp falling off of responses following the maximum. This falling off was at first more rapid than the fall of responses at 3 or 4 but tailed off and stopped when values equal to or less than those at the start of asphyxia were reached. The new level was not attained until after recovery at 5 and 6 was complete, but usually some time before 3 and 4 had returned to their base line.

A number of experiments were made in attempting to account for the peak at 1 and 2—the subsequent fall to original levels is largely the reverse of the rise during asphyxia, and need not especially concern us. Diffusion of substances along the nerve producing local changes of the upper electrodes can easily be ruled out. An "oxygen lack" could hardly spread to nerve in oxygen, and unoxidized products diffusing from the asphyxiated region, even if not oxidized, should affect electrode 2 more than 1. Also, diffusion should be equal to 5 and to 2, but the former does not show this behavior. Finally, the changes are too rapid to represent ordinary diffusion and occur simultaneously at 1 and 2. The only other way apparent in which the nerve above the nitrogen region could be locally modified involves "distance action" via electrical circuits.

The injury potential of asphyxiated nerve falls greatly (see later), so that in effect the "killed end" is moved to the proximal portion of the nitrogen region. Local currents must flow from here through the axone "cores" and back on the outside of the fibres or sheath, as previously discussed.

Responses near the region of asphyxia should then be depressed, as near a crush, and the rise at 2 during the asphyxiation period should be less than normal. Restoration of membrane potentials in the nitrogen stretch would then increase responses at 2 until returning activity at the end lead, 7, again cut them down. This factor may play a rôle, but on this basis the changes at 2 should again be more pronounced than at 1, which is not the case. Polarizing the nerve at lead 2 (and 3) with potentials of the same strength as injury potentials also failed to affect the action potentials at 2, either during passage of the polarizing currents or at the time of closing or opening.

If the nerve at leads 1 and 2 is not locally changed, the electrical response may still be modified in at least two ways. The path of local current flow in the nerve during conduction of an impulse could be modified, or the physical conditions of the recording circuit might be changed. As regards the former, several possibilities can be conceived, but are simply excluded by sending impulses along the nerve in the reverse direction. If, for *A* stimulation, these effects appear at 1 and 2 but not at 5 and 6, then for *B* stimulation they should not appear at 1 and 2 on the far side of the asphyxiated stretch. Actually, however, they still appear at 1 and 2 when stimulation is carried on at *B*.

This leaves as the necessary condition for the appearance of a peak outside of an asphyxiated region, that such a region must be interposed between the lead on normal nerve and the "dead" lead. The position of the stimulating electrodes and the direction of the impulse are not crucial. The interposed asphyxiated stretch could not produce effects by any change in longitudinal resistance, as previously pointed out. Since the peaks above it do not occur during the asphyxiated period but afterwards, when the asphyxiated nerve is showing its own peak response, the former appears to be somehow dependent on the latter. It is possible that the idle electrodes on the nitrogen stretch are acting as secondary leads back to those above, as found to occur by Bishop, Erlanger and Gasser (1926), though even here it is not clear why 1 should be as much affected as 2. This effect depends on local lowering of the transverse resistance and it could well occur even with no electrode contacts if asphyxia interferes with the membrane's integrity. On this interpretation, the high responses at 1 and 2 depend on increased responses and a persisting low membrane resistance in the previously asphyxiated stretch, and since both factors are changing after the peak to lessen this effect, the falling off of responses after the peak at 1 and 2 should be more rapid than at 3 or 4, as it is. A further indication that responses at the proximal leads are due in part to back currents from the asphyxiated region comes from the F/S ratios. These fall during the peak at 1 and 2 nearly as low as at 3 and 4, as should occur on this assumption, but could hardly result if the responses at 2 measured the

nerve activity at 2. On the other hand, the peaks at 1 and 2 have not always been synchronous with those at 3 and 4, and 1 and 2 have even been well past their peak responses before those at 3 have been reached. It is not clear how far this "back leading" can be invoked to explain the peaks in the unasphyxiated stretch. It may be added that a similar effect is seen with CO_2 in place of N_2, and it appears even when no metal leads are in contact with the nerve in the exposed region.

One experiment with a bull frog nerve mounted in the reverse direction so that only a portion of the fibres were stimulated at *A* yielded unique results (fig. XI). There can be little doubt here that responses at 1 and 2 varied intimately with those at 3 and 4 but this time in the *opposite*

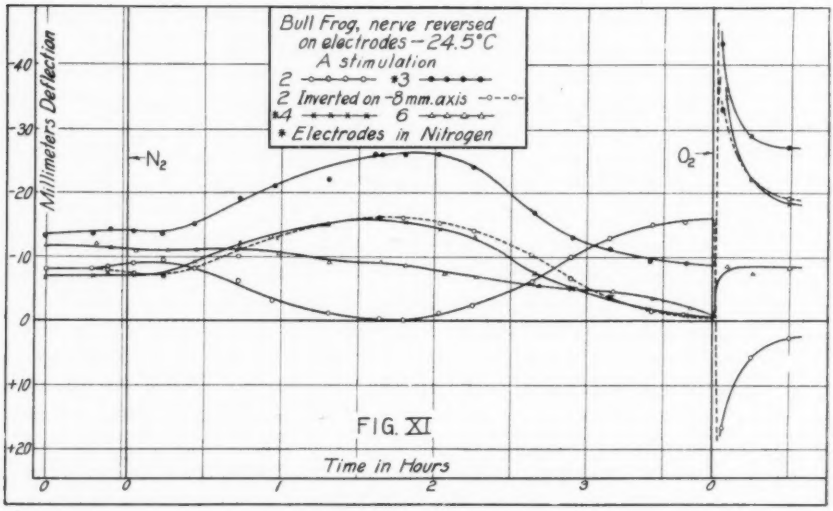

Fig. XI

direction. The curve at 2 is an almost perfect mirror image of 4, including a fall in nitrogen during the rise of 4, a subsequent greater rise as 4 fell, an inverted peak with positive action potentials when oxygen was readmitted and eventually a return to the initial values. It is difficult to fully interpret this curve, but it might be surmised that the apparent positive peak at 2 is really a negative peak at 7. As usually mounted, many fibres run past leads 1 and 2 to leave the nerve as a branch as it enters the nitrogen compartment. With the reversed nerve, this is the case for lead 7. The nerve branch thus appears to have some relation to the outside peak. The peaks outside of the asphyxiated region occur, however, in the unbranched dog peroneal, so branching is not the essential factor.

Whatever the true basis for this distal effect of asphyxia (and the same appears with carbon dioxide), it is of considerable interest. It may seriously complicate the picture in experimental studies. The present set of observations has been carefully scrutinized for possible errors of interpretation due to it, and I believe they do not occur. Further, it may be a clue to a mechanism, other than the passage of nerve impulses, whereby one region of the nervous system can affect another. The work of the Pavlov school, the Gestalt psychologists (as Lashley, 1929) etc. strongly suggests that such an additional mechanism does exist.

6. Resting potentials. These were measured directly by the balancing potential required between any two electrodes or indirectly by the galvanometer throw on changing from one electrode to another, with the condenser circuit. The results were similar with either method, though

TABLE 10

LEADS	CRUSH ABOVE 7—24 HOURS PREVIOUSLY		FRESH CRUSH BELOW 3	
	Resting potential	Action potential	Resting potential	Action potential
	volt	*mm. deflection*		
1–7	+0.008	57	−0.003	162
2–7	+0.006	37	+0.005	155
3–7	+0.026	39	−0.011	5
4–7	+0.028	35	+0.046	0
5–7	+0.012	44	+0.006	0
6–7	+0.000	23	+0.017	0
1–4	−0.020	22	−0.055	170
3–4	+0.001	4	−0.057	6

Bull frog nerve, 24 hours after dissection. T° = 22°C. First observation 12 hours after a five hour asphyxiation of the region on leads 3 and 4.

the former is, of course, more exact. The resting potentials at different points along the side of an uninjured, unbranched nerve (measured against any one point) are seldom equal and may vary widely. The potential difference between two leads from the intact side may, in fact, be considerably greater than between side and end (table 10). Presumably local injury, tissue tags, relation to injured end, etc., are responsible for these differences, though following a careful dissection there are no gross irregularities and test may show nearly all fibres able to conduct their whole length. The magnitude of the action potential from any pair of leads has no relation to the resting potential between them. Weak action potentials may be obtained from two leads on the side of an unbranched nerve showing large or small resting potential differences, and action potentials of the usual strength may appear on leading from side and end even though the resting or injury potential between them is practically zero. Since in

these experiments the nerve was crushed several millimeters above the lower electrode, the portion lying on it was not severely injured, and this independence has not the full significance it would otherwise have. A crush does, however, influence the potentials at nearby points, for lead 7, near the crush, regularly is at a lower voltage than the other leads, and crushing at any point markedly alters the resting potentials of regions near it.

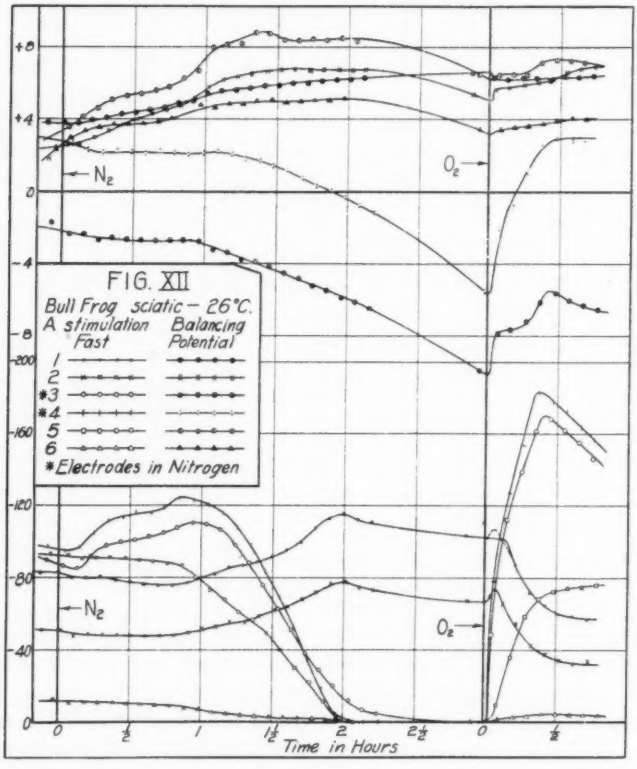

Fig. XII

These resting differences are small compared to those appearing regularly (but not invariably) in the course of asphyxia. The resting potentials at the electrodes in the nitrogen stretch fall steadily (become more negative) during the course of asphyxia and rise, more rapidly, on the readmission of oxygen (fig. XII). The potentials reached after full recovery in oxygen may be definitely higher than at the start of the asphyxia, but they

are then maintained with no evidence of a "peak" effect. The fall and return of resting potential parallels the changes in action potential rather strikingly in some experiments; but in one case, despite a typical failure of action potentials, the resting potentials at 3 and 4 (against 7) showed a steady slow rise unaffected by the asphyxial period. I have no explanation for this anomalous result.

The fall in resting potential during asphyxia is in harmony with current views of nerve activity, and with Furusawa's findings on crab nerve (1929). The resting fibre is supposed to possess a longitudinal membrane polarized as an electrical double layer, positive outside and negative within. The injured region is negative to the intact one because this insulating membrane is destroyed, and an active region becomes negative for the same reason. The polarized membrane is a living structure and presumably maintains its integrity by the continual expenditure of energy obtained by oxidations. This has been suggested by Warburg (1912) in the case of nucleated erythrocytes and by Hill (1928) for the case of muscle. For nerve, Gerard (1927) found an extra oxygen consumption after a period of asphyxia, part of which reappears promptly as CO_2 (Fenn, 1928) and represents increased oxidations. Since it has not been possible to demonstrate an accumulation in nitrogen of metabolites which are subsequently oxidized (Gerard and Meyerhof 1927; Holmes and Gerard, 1929), it seems probable that much of the extra oxidation goes to the rebuilding of the cell membrane. Evidence of a decreased membrane resistance during asphyxia has been presented earlier in this paper. The resting potential, then, measures a dynamic equilibrium point of disintegrative and restoring reactions.

The fall of injury potential in nitrogen is thus additional evidence that the normal membrane potentials (and other properties) are dependent on oxidations. That they arise at the intact portion of the cell rather than at the injury is further evidenced by the finding that the asphyxial fall is the same whether the injured portion is included in nitrogen or is left in oxygen.

If activity represents membrane destruction (Lillie, 1923) and the delayed potentials signal its incomplete restoration, the progressive failure of recovery in nitrogen must lead to ever greater[4] delayed potentials or, in other words, failure of recovery of the resting potential. Fibre block may then be one expression of the failure of membrane restoration due to lack of oxygen, the fall of resting potential being another. A similar view has been expressed by Furusawa (1929) in interpreting the "total depolarization" of crab nerve when fatigued to inactivity. This interpretation

[4] Greater in the sense of farther from the initial value to which the potential normally would return. This would be true for the summed delayed potentials. The added effect of a single impulse must ultimately become less as the resting potential falls and so reduces the change possible on activity.

does not imply that the chemical reactions of rest and of activity are the same, and in fact they are not (Gerard and Meyerhof, 1927; Holmes, Solomon and Gerard, 1930).

The thesis developed in this paper is simply an effort to satisfactorily correlate recently acquired facts concerning thermal, chemical and electrical events of nerve function. It is in no sense a new theory of nerve activity but does serve to indicate certain further experiments.

SUMMARY

1. Resting and action potentials of several types of nerve have been studied by means of a slow galvanometer, using multiple leads and two frequencies of stimulation applied at two regions. The use of a condenser in the galvanometer circuit was convenient and allowed reliable measurements. A condenser was also used across the primary of the stimulating circuit, and its capacity found to have a marked influence on the effectiveness of the shocks.

By comparison of responses at electrodes in and below nitrogen to stimuli at high or low frequency it is possible to obtain information as to: total action potential per fibre-impulse, per cent of fibres blocked, prolongation of refractory period, changes in form and intensity of action-potential.

2. The action potentials led from nerves before asphyxiation are considerably greater for the green frog than for the bull frog or dog. All leads from the intact side, even with an unbranched nerve, do not give equal responses. Potentials from the lead nearest the crush (15 mm.) are regularly depressed, even to half the values at the other leads.

Responses to rapid tetanization (300 per sec.) compared to slow tetanization (90 per sec.) are not as much greater as the frequency ratio of the stimuli (F/S = 3.5), but definitely less, even by half (F/S = 2.0–3.0).

Responses fall rapidly for a short time after a crush is made and then very slowly. After 24 hours, a fresh crush will often restore action potentials to their original value. Evidence is presented that the early rapid fall is due to increasing activity at the crush and consequent greater diphasicness. Due to this activity at the crush, which may reach three-fourths of the normal, depression of activity at other electrodes may give reversed responses from leads on the "intact" side and "dead" end.

When make and break stimuli are applied to a nerve between the leads, unidirectional potentials develop. This effect (Fleischl) apparently depends on rectification by the nerve membranes and is some measure of their physical state. The Fleischl effect is greatly reduced in asphyxia.

There is regularly present a positive potential change following the negative action potential; a positive after-effect. This is abolished during asphyxia.

The rise of total action potentials (equilibration) occurring early with

frequently repeated tetanization is greater for fast than for slow tetanization and may be abolished or reversed during asphyxia.

3. In the course of asphyxia, action potentials led from the exposed region first rise and then fall to zero. Potentials led from nerve in oxygen beyond the asphyxiated stretch remain constant during the rise of the others and then fall with them. The fall usually shows definite waves. During the fall, responses at the distal electrode in nitrogen fall faster than at the proximal and may reach values only half as great as in the control stretch below. Responses to fast tetanization fall faster than to slow and a Wedensky effect may appear, in which the nerve below the asphyxiated region still responds when slow tetani are delivered above the nitrogen stretch, but shows no response to fast tetanization. The mechanism of nerve block is discussed.

These observations are interpreted to mean that asphyxia causes: first a prolongation of action potentials, later a depression of potential magnitude, a prolongation of refractory period, and fibre block. Fibres do not block in a homogeneous manner but fall in groups, probably those of each diameter forming a group.

Asphyxia blocks impulses and abolishes potential changes at the point of block—like a fresh crush; but activity at the block (diphasicness) does not gradually return as in the case of a crush.

4. Resting potentials are steadily lowered (side less positive) in an asphyxiated stretch and return in oxygen. The relation of the membrane potentials to action potentials, and the rôle of oxygen in maintaining them is discussed; and an interpretation is offered for activity at a crush and depressed resting and action potentials and increased irritability near it.

5. The time required for asphyxiation of a nerve depends on the temperature and the type of nerve. Dog nerve asphyxiates more rapidly than green frog and this more rapidly than bull frog. The resting oxygen consumption of these nerves is in relation to the time for asphyxiation—the higher the metabolism the faster the block. This is interpreted in terms of exhaustion of an oxidizing reserve. The failure of fibres in groups is similarly interpreted.

6. When oxygen is readmitted to an asphyxiated nerve the action potentials in the exposed region rise rapidly (5 to 10 minutes) to very high values. For fast stimulation, the maximum response may be 5 or 6 times as great as the pre-asphyxial ones, for slow stimulation even 12 times as great. The maximum is a "peak" value and is followed by a less rapid fall to or towards the initial values.

Responses below the nitrogen region show no peak but return in about 15 minutes to their original values.

Nerves kept practically free of Ringer's solution from the time of their dissection often recover fully in oxygen and remain in good condition over

night. Asphyxiated nerves bathed in oxygen free Ringer usually show no recovery. The theory that asphyxial block results from the accumulation of metabolites is considered and discarded. The changes in nitrogen and oxygen are interpreted in terms of two opposed effects due, possibly, to separate chemical reactions.

7. Electrodes above the asphyxiated region, reached by impulses travelling only in normal nerve, record increased responses as the asphyxia below progresses. The rise starts simultaneously with the fall of the electrodes below nitrogen, and at first the ratio of fast over slow increases. This effect is due to the more perfect monophasicness of nitrogen block than at a crush.

The upper electrodes also show a further peak in responses paralleling the peak in the previously asphyxiated region. Its causation is uncertain, but may represent a back lead from the recovering stretch.

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